UNIVERSTIÉ DU QUÉBEC À MONTRÉAL

ÉTUDE DES FACTEURS PÉDOCLIMATIQUES ET NUTRITIFS RÉGISSANT LA CROISSANCE DE *SALIX MIYABEANA* SX67

THÈSE

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AVANT-PROPOS

Cette thèse est composée de trois chapitres. L'introduction et la conclusion générales sont rédigées en français alors que les chapitres sont en anglais et présentés sous forme d'articles scientifiques. Comme les articles n'ont pas été formatés pour les mêmes revues, le format du résumé des chapitres 1 et 2 (« Abstract ») diffèrent de celui employé pour les chapitres 3. À ce jour, le premier chapitre a été soumis à la revue BioEnergy Research.

RÉSUMÉ

Les cultures intensives à courtes révolutions (CCR) de *Salix* peuvent être mises à profit à des fins diverses, comme la production de biomasse, la phytoépuration, la phytoremédiation et la séquestration du carbone dans le sol. L'établissement de CCR constitue également une alternative à la valorisation des terres marginales au Québec. Le cultivar *Salix miyabeana* SX67 est reconnu parmi les plus productifs. L'objectif de cette thèse était d'étudier et de quantifier à l'échelle du Québec méridional les impacts du climat et des propriétés physico-chimiques du sol sur la productivité épigée. Il était aussi souhaité de quantifier ces relations par l'entremise de la nutrition foliaire.

Cette étude a été réalisée en mettant à profit dix CCR établies dans le sud du Québec, soumises à des conditions pédoclimatiques hétérogènes. Des analyses physico-chimiques ont été effectuées sur les sols de chaque site. Les teneurs des éléments dans les feuilles ainsi que la masse foliaire ont été mesurées à différents sites au cours des saisons de croissance de 2011 à 2013. Le $\delta^{13}C$ foliaire a aussi été évalué au cours de l'année 2011. Enfin, les climats annuels ont été modélisés pour chaque site à l'aide du modèle BioSIM.

La croissance de SX67 a été quantifiée en tenant compte de l'effet du recépage. De ce projet, il résulte une base de données générée à l'aide de mesures effectuées entre 2001 et 2013 sur plus de 1100 arbres ayant des âges d'appareils racinaires et de tiges compris entre 1 et 15 ans et 1 et 7 ans, respectivement. Des analyses dendrochronologiques ont également permis d'effectuer un suivi de la productivité annuelle durant minimalement le dernier cycle de chaque CCR. Ainsi, il a été mis en évidence qu'après un laps de temps nécessaire à l'établissement de l'arbuste (compris entre un minimum de deux ans sur sol sableux et un maximum de quatre ans sur sol argileux et compacté), le potentiel de productivité maximal était atteint et a pu être estimé. Le gain de productivité entre les deux premiers cycles était relatif à la durée de ce délai et donc aux propriétés du sol.

Certaines propriétés du sol telles que les teneurs en limon et en magnésium échangeable ont expliqué significativement 72% et 25% des variations entre les sites, respectivement. Uniquement sur sol minéral, le limon, le calcium échangeable et le carbone organique ont expliqué 72%, 54% et 41% des variations. Au sein de chaque site, les facteurs limitants ont différé relativement aux statuts nutritionnels des sols et à leurs propriétés physiques. Sur un site, l'historique agricole a engendré des conditions pédologiques qui confinent les racines proches de la surface du sol. Ceci a engendré un stress hydrique accru, ce qui a été mis en évidence par les valeurs foliaires de $\delta^{13}C$. Également, les variations inter-annuelles de sécheresse estivales étaient négativement liées à la productivité, sauf pour un site où il n'a pas semblé y avoir eu de limitation en eau. Exceptionnellement, à cette CCR, la relation entre la précipitation et la productivité était négative. Globalement, la sécheresse a expliqué

significativement 10 à 20% de la productivité entre les sites, alors que les propriétés du sol ont expliqué environ 70%.

Bien qu'une signature nutritionnelle foliaire spécifique à chaque site ait été mise en évidence, les fluctuations climatiques inter-annuelles ont eu des répercussions importantes sur la nutrition foliaire. Les variations de nutrition inter-annuelles ont été observées et, en partie, expliquées par certaines variables climatiques. De ce fait, les nutriments foliaires linéairement liés à la productivité variaient selon les années. Dans l'ensemble, l'azote, le calcium et dans une moindre mesure le manganèse foliaires étaient les plus fortement liés au rendement. Toutefois, l'utilisation d'arbres de régressions multivariés a permis d'expliquer la productivité annuelle par des seuils nutritionnels et ainsi de passer outre les limitations spécifiques aux années.

Cette étude suggère de modifier certaines pratiques telles que le recépage initial ou le labour avant l'établissement d'une CCR. Bien que la productivité de chaque CCR soit limitée par des facteurs leur étant spécifiques, la sécheresse et l'acidité des sols sont les principaux facteurs limitants dans le sud du Québec.

MOTS CLÉS : Saule, nutrition foliaire, nutriments du sol, biomasse, culture à courte révolution, fluctuation climatiques annuelles

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ABI = Abitibi

ALB =Albanel

BEL = Beloeil

BOI = Boisbriand

CND = Compositional Nutrient Diagnosis

CFN = Concentration of Leaf Nutrients

HTG = Huntingdon

LAV = Laval

MRT = Multivariate Regression Tree

MTL = Mont-Laurier

MYP = Maximum yield potential

RXP = Roxton Pond

SJPJ = Saint-Jean-Port-Joli

SRC = Short rotation intensive culture

STR = Saint-Roch-de-l'Achigan

INTRODUCTION GÉNÉRALE

1.1 Le saule

Le saule (*Salix spp.*) est utilisé à des fins très diverses depuis des millénaires par les Égyptiens, les Romains et les Occidentaux (par ex. usage médicinal, fabrication de paniers et de flûtes, stabilisation des berges, (Keoleian 2005)). L'utilisation de *Salix* en régime de production intensive à des fins bioénergétiques a connu un engouement en Suède dans les années 70 (Christersson *et al.* 1993). Bien qu'encore en développement, les utilisations sont aujourd'hui également destinées à d'autres usages tels la phytoremédiation (Kuzovkina & Volk 2009), la phytoépuration (Mirck & Volk 2010), la phytoextraction (Meers *et al.* 2007) ainsi que la revalorisation des boues d'épuration (Labrecque & Teodorescu 2001; Kuzovkina & Volk 2009) et du lisier de porc (Cavanagh *et al.* 2011). Avec la contribution d'un amendement adéquat, les cultures de *Salix* ont l'avantage de pouvoir être élaborées sur des terres marginales (Christersson 1986), tout en améliorant les propriétés du sol (par ex. réduction de l'érosion, amélioration des propriétés de filtration, capacité de décompaction du sol et de stabilisation de la structure du sol (Kuzovkina & Volk 2009)). De plus, il a été démontré que la biodiversité dans les cultures de *Salix* est accrue comparativement aux cultures agricoles conventionnelles ou aux cultures de conifères monospécifiques (Perttu 1999). Également utilisables à des fins de production de biocarburant de seconde génération (Smaliukas *et al.* 2007), il est aujourd'hui indéniable que les cultures à courtes rotations (CCR) sont une alternative très prometteuse quant à la production d'énergie (Volk *et al.* 2004). De surcroît, les CCR permettent d'augmenter sensiblement la teneur en carbone organique séquestré dans le sol (Lockwell *et al.* 2012; Routhier *et al.* 2014; Lafleur *et al.* 2015). *Salix* est un genre de début de succession. Caractérisés par une croissance rapide, plusieurs espèces et cultivars de *Salix* profitent d'une saison de croissance relativement courte. Ainsi, de nombreux cultivars de *Salix* peuvent être cultivés sous les conditions

pédoclimatiques du Québec méridional (Labrecque & Teodorescu 2003; Guidi Nissim *et al.* 2013).

0.2 Les cultures à courtes révolutions et le recépage

Pour mettre en place une CCR, des boutures (section de tige d'environ 20-25 cm généralement prélevée après un an de croissance) sont plantées à une profondeur d'environ 18 cm (Guidi *et al.* 2013). Bien que la densité soit variable, les CCR au Québec comptent en général entre 18 000 et 20 000 boutures par hectare (Labrecque & Teodorescu 2003; Labrecque & Teodorescu 2005; Guidi Nissim *et al.* 2013). Les tiges qui émergent sont généralement récoltées après 3 à 5 ans (Mola-Yudego & Aronsson 2008), bien que cela puisse varier de 2 à 8 ans selon les rendements des sites (Guidi *et al.* 2013). Les souches restantes produisent alors de nouvelles tiges qui seront récoltées à nouveau à la fin de cycle suivant et ainsi de suite. Il est usuellement accepté qu'une CCR peut-être exploitée une trentaine d'années.

L'effet du recépage sur la croissance de *Salix* n'est pas anodin. En fin de première année, un recépage initial est fréquemment effectué dans le but d'augmenter le nombre de tiges et la biomasse finale de la première révolution (Guidi *et al.* 2013). Également, il a fréquemment été rapporté que le rendement de la deuxième révolution était supérieur à celui de la première (Nordh 2005; Mola-Yudego & Aronsson 2008; Guidi Nissim *et al.* 2013). De plus, les CCR n'ont pas un nombre maximum de révolution établi. De façon anecdotique, certains auteurs ont même observé une augmentation de productivité jusqu'à la 4ème révolution (Volk *et al.* 2011). Avant que la production épigée soit maximale, un laps de temps est nécessaire pour que l'appareil racinaire soit suffisamment bien établi (Kopp *et al.* 1993; Mola-Yudego & Aronsson 2008). Kopp *et al.* (2001) ont observé une augmentation asymptotique de la productivité au cours des deux à quatre premières saisons de croissance, selon que le sol ait été fertilisé ou non. Toutefois, la durée de ce délai en fonction de la variabilité des sols n'est pas encore élucidée.

De la même façon, peu d'études ont porté sur l'effet du recépage sur la productivité en fonction de la variabilité des sols (Abrahamson *et al.* 2002) et à ma connaissance, aucune recherche n'a porté sur les effets du recépage initial.

0.3 Les conditions de sol régissant la nutrition et la croissance de *Salix*

Très adaptable, *Salix* peut croître quelque soit la texture granulométrique du sol (Tahvanainen & Rytkönen 1999; Labrecque & Teodorescu 2001), y compris sur sol compacté (Kuzovkina *et al.* 2004; Souch *et al.* 2004). Cependant, la productivité est supérieure sur les sols à textures fines, bien que les sols argileux compactés ne soient pas non plus optimaux (Abrahamson *et al.* 2002). Sur les sols sableux et caractérisés par de faibles teneurs en azote (N), les rendements sont plus faibles (Labrecque & Teodorescu 2001; Toillon *et al.* 2013). En culture hydroponique, les rendements augmentent avec la disponibilité en N (Ericsson 1981a). Cependant, *in situ*, les réponses sont très variables selon la nature des sols (Labrecque & Teodorescu 2001; Quaye & Volk 2011; Simon *et al.* 2013). L'effet des autres nutriments n'a été que peu étudié. En culture hydroponique, il a été démontré que le N, le phosphore (P), le potassium (K), le calcium (Ca) et le magnésium (Mg) sont positivement liés à la croissance (Ericsson 1981b). En revanche, *in situ*, les relations sont beaucoup moins triviales et les conclusions ne sont pas unanimes (Simon *et al.* 1990; Labrecque *et al.* 1998). En effet, les facteurs limitants peuvent varier d'un site à l'autre. En sol acide, les métaux sont plus disponibles (Sanders 1983; Martínez & Motto 2000) et sont susceptibles d'être toxiques pour les arbres ainsi que d'induire des effets antagonistes aux prélèvement d'autre nutriments. Par exemple, dans de telles conditions, il a été observé que le manganèse (Mn) limitait la croissance de *Populus*, de *Acer saccharum* et de *Salix* (Kovalchik 1992; Houle *et al.* 2007; Pinno *et al.* 2010). De plus, les sols acides sont généralement carencés en Ca, ce qui peut également limiter la croissance de *Salix* (Hytönen 2005). Pour la majorité des cultivars, il semble qu'une gamme de pH optimale se situe entre 5-5.5 et 7-8 (Mitchell *et al.*

1999; Abrahamson *et al.* 2002; Guidi *et al.* 2013). Cependant, une relation positive a été observée (sans seuil maximal) entre le pH du sol, le Ca total du sol, le K foliaire et la productivité de *Salix purpurea* sur plusieurs sites ayant des valeurs de pH de sol allant jusqu'à 8 (Ens *et al.* 2013). Concernant le cultivar *Salix miyabeana* SX67, sa sensibilité au pH ainsi que les conditions de nutrition optimales restent à définir.

Les teneurs nutritionnelles des sols se reflètent dans les feuilles plus ou moins linéairement (Bauer *et al.* 1997) et dépendent du mode d'absorption des nutriments (Ericsson 1981b). Ainsi, l'augmentation du rendement de *Salix* après l'ajout de fertilisants à base azotée et de chaux se traduit respectivement par une augmentation du N foliaire (Labrecque *et al.* 1998; Labrecque & Teodorescu 2001) et du Ca foliaire (Hytönen 2005). Également, les nutriments sont susceptibles de rentrer en interaction. Par exemple, le ratio Ca:Mg influence l'absorption en plomb (Pb) et en cadmium (Cd) qui, en retour, limitent la croissance de *Salix* (Mleczek *et al.* 2011; Magdziak *et al.* 2013). De ce fait, afin de mieux détecter ce type d'interaction, différentes méthodes de diagnostique foliaire ont été élaborées pour mieux interpréter les teneurs nutritionnelles brutes obtenues (Bates 1971; Beaufils 1973; Parent *et al.* 1994a; Haase & Rose 1995; Parent 2011). Dans un espace contraint comme celui d'une feuille, la variation de la teneur d'un nutriment a forcément un ou des impact(s) sur la ou les teneur(s) d'un ou des autre(s) nutriment(s). Il y a donc une redondance dans l'information contenue dans les teneurs brutes des nutriments foliaires parce qu'elles ne peuvent pas être considérées comme indépendantes. Afin de surmonter ce biais, la méthode du « Compositional Nutrient Diagnosis » (CND) permet de calculer, pour chacun des nutriments, des indices linéairement indépendants qui prennent en considération le bilan nutritionnel total de la feuille ou de l'aiguille (Parent & Dafir 1992). Bien que cette méthode n'ait pas encore été utilisée pour un cultivar de *Salix*, elle semble tout à fait adaptée au contexte de cette étude.

0.4 Le climat comme facteur limitant la croissance de Salix

Bien que *Salix* soit très adaptable selon une large gamme de températures et de régimes hydriques (Donovan *et al.* 1988), les contraintes climatiques sont susceptibles de limiter la croissance dépendamment de l'aire de l'étude. Dans les zones géographiques les plus froides, la température estivale et la longueur de la saison de croissance sont généralement limitantes (Wijk 1986; Walker 1987; Schmidt *et al.* 2010). Dans les zones plus tempérées, la température est positivement liée à la croissance des arbres s'il n'y a pas de limitation hydrique, alors que la relation s'inverse en cas de sécheresses (D'Arrigo *et al.* 2008). Ces tendances se vérifient bien sur *Salix*. Dans la vallée du Po en Italie, où de fortes sécheresses estivales caractérisent le climat (mésogéen), la pluviométrie a été identifiée comme facteur limitant le rendement de *Salix matsudana* (Bergante *et al.* 2010). En revanche, sous un climat n'imposant aucune limitation hydrique comme sur l'île de Svalbard (Norvège), par exemple, la pluviométrie est négativement liée à la croissance de *Salix polaris* car elle diminue la température et la radiation (Buchwal *et al.* 2013). Aussi, au sud du Québec, la productivité maximale de *Salix* en CCR (au-delà de 20 t ha^{-1} an^{-1}) est supérieure à celle du sud de la Scandinavie si les plantations ne sont pas irriguées (Christersson 1987; Tahvanainen & Rytkönen 1999; Labrecque & Teodorescu 2003), alors que le nombre de degrés jours (base 5°c) est environ le double et que la pluviométrie de mai à aout est deux fois moindre (Labrecque & Teodorescu 2003). De ce fait, les faibles précipitations ont déjà été soupçonnées de limiter la productivité dans le sud du Québec, bien qu'à ce jour, aucune étude n'a quantifié l'ampleur de l'effet.

Le ratio ^{12}C:^{13}C dans les tissus de la plante est un indicateur robuste de l'efficacité de l'utilisation de l'eau au cours de la saison de croissance (Farquhar *et al.* 1982). Cette information est précieuse parce que les exigences en eau des cultivars de *Salix* cultivés en CCR sont très élevées (Weih 2001). Chez *Salix viminalis*, 6,3 g de matière sèche par kg d'eau transpirée est, en moyenne, produite (Lindroth 1996). Combinée aux rendements, l'analyse du $\delta^{13}C$ (signature isotopique du ^{13}C) a permis de mettre en évidence des sites où un stress hydrique avait fortement

limité la productivité et, de manière plus nuancée, de constater si l'efficacité de l'utilisation de l'eau limitait la productivité de diverses CCR de *Salix* (Moukoumi *et al.* 2012; Ens *et al.* 2013; Toillon *et al.* 2013).

En même temps que la croissance, les conditions climatiques annuelles modulent la nutrition des arbres (MacKay & Leefe 1962). Notamment, les teneurs en N foliaire baissent durant les années de faibles pluviométries et/ou de fortes températures (Kudo 2003; Vizcayno-Soto & Côté 2004). Toutefois, très peu d'information est disponible sur le sujet, encore moins sur le *Salix*. De nombreuses interrogations demeurent quant aux effets des interactions entre les propriétés pédologiques et les conditions climatiques sur le statut nutritionnel de la feuille et sur le potentiel de croissance de *Salix*.

0.5 Objectifs, hypothèses et structure de la thèse

L'objectif de ce doctorat est de mieux comprendre et de quantifier l'influence des facteurs pédoclimatiques et nutritifs régissant la production de biomasse épigée de *Salix miyabeana* SX67 dans dix CCR dispersées au sein du Québec méridional. *Salix miyabeana* est une espèce qui pousse naturellement dans les forêts de Hokkaido, Japon, (Kudo 2003). Le cultivar *Salix miyaneana* SX67 a été sélectionné pour cette étude car son potentiel de productivité est parmi les plus élevés relativement aux autres cultivars de *Salix* et il est aussi plus résistant aux maladies et aux insectes que *Salix viminalis*, également reconnu pour ses rendements élevés au Québec et en Amérique du Nord en général (Labrecque & Teodorescu 2005; Guidi Nissim *et al.* 2013). De manière à apprécier au mieux cette question de recherche, trois chapitres comprenant chacun des objectifs et des hypothèses spécifiques qui traitent de cette question sous différents angles ont été rédigés.

Les objectifs du premier chapitre étaient (1) de reconstruire la productivité annuelle de biomasse épigée durant minimalement le dernier cycle de révolution des dix CCR dont les âges racinaires et les historiques de recépage sont différents,

(2) de comparer le potentiel de productivité des sites et (3) de mettre en évidence l'effet du recépage sur la biomasse sèche, le diamètre basal et la hauteur de la plus grosse tige ainsi que le nombre de tiges en fonction des propriétés du sol de chaque site. Pour ce faire, la signification de la relation linéaire entre la surface basale de la tige de plus gros diamètre et la biomasse sèche a été testée. Ensuite, via des analyses de dendrochronologie, les dynamiques de croissance annuelle ont été étudiées afin de dégager les influences de l'historique de recépage ainsi que celles des conditions de site. Enfin, l'effet de l'âge du système racinaire sur la productivité a été testé à différents sites en comparant la productivité de tiges de mêmes âges récoltées sur des souches d'âges différents.

Les objectifs du deuxième chapitre étaient (1) de comparer et de quantifier les effets intra- et inter-sites des conditions pédoclimatiques sur le rendement et (2) de développer des modèles pédoclimatiques afin de comparer les effets du sol et du climat. Le but ultime était de pouvoir estimer le potentiel de productivité d'un site en fonction de variables simples afin d'éclairer le plus possible les producteurs sur le potentiel de leurs terres en vue de l'établissement éventuel d'une CCR de *Salix*. En conséquence, toutes les variables pédoclimatiques ont été testées afin de voir si elles étaient en mesure d'expliquer significativement la productivité. Également, l'hypothèse que tous les sites soient limités par les mêmes contraintes climatiques a été testée. Finalement, il a été testé si les rendements annuels pouvaient être expliqués par des seuils de variables pédoclimatiques.

Les objectifs du troisième chapitre étaient (1) de quantifier dans quelle mesure les variations des équilibres nutritionnels foliaires inter-sites et inter-annuels sont imputables aux conditions édaphiques et climatiques et (2) de développer des modèles capables de prédire le rendement annuel à l'aide de variables foliaires. Pour ce faire, il a été testé si une signature nutritionnelle foliaire spécifique au site peut-être mise en évidence, indépendamment des fluctuations nutritionnelles annuelles. Ensuite, il a été testé si les nutriments foliaires étaient linéairement liés aux variables climatiques, pédologiques et à la productivité annuelle. Finalement,

il a été testé si des modèles basés sur des effets seuils étaient capables d'expliquer la productivité.

CHAPITRE I

EFFECTS OF COPPICING AND STOOL AGE ON ANNUAL YIELDS OF SALIX MIYABEANA SX67 IN SOUTHERN QUÉBEC

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1.1 Abstract

Aboveground biomass yields of short rotation cultures (SRC) of willow can vary substantially depending on site quality. Among others, aboveground biomass yields depend on climatic conditions, soil properties, age of the SRC and number of harvesting cycles. In this study, we investigated the effects of coppicing on growth variables (i.e. largest basal stem, height and aboveground biomass) at ten SRC of Salix miyabeana SX67 established on various soils in southern Quebec. More than 1100 shrubs with stool ages varying between one and fifteen years were measured. Strain analysis was carried out to calculate past annual aboveground productivities, and maximum annual yield potential was quantified at each site. Annual growth rates were highly variable and depended on site and coppicing history. To achieve optimal stool development and aboveground yields, two to three growing seasons following coppicing are necessary for sandy and clayey sites, respectively. The delays for reaching maximum yields were shortened when soil cation exchange capacity was dramatically low and were prolonged when soil was physically restricting stool development. This lag influenced the total yield of the first rotation and also modulated the magnitude of the increase of aboveground biomass that is generally observed in the second rotation. To increase yields in southern Quebec, our results suggest that it is preferable to extend the length of the first rotation instead of coppicing at the end of the first growing season after establishment.

Keywords: Willow; short rotation culture, annual yield, soil texture, coppicing, root system age

1.2 Introduction

The use of willows (*Salix* spp.) is considered for various ecological applications such as bioenergy (Volk *et al.* 2004; Karp & Shield 2008; Guidi Nissim *et al.* 2013), phytoremediation (Weih & Nordh 2002) and waste water and sludge filtration (Labrecque *et al.* 1998; Dimitriou *et al.* 2006; Cavanagh *et al.* 2011). Willows can colonize and perform relatively well on various soil types, which makes them an interesting option for landowners with unused marginal land (Christersson 1986; Karp & Shield 2008). Short rotation culture (SRC) of willow has recently grown in interest in Quebec as yields of *Salix viminalis* planted at a density of 18 000 stools ha^{-1} in the warm southern region were reported to be as high as 70 Mg ha^{-1} of dry weight (DW) after a second rotation of three years (Labrecque & Teodorescu 2003). This cultivar is also known for its high potential productivity in other regions with similar temperate climates [e.g., Verwijst (1996), Bergkvist and Ledin (1998) in Sweden, and Stolarski (2008) in northern Poland], although yields in southern Quebec appear to be in the upper tier of all yields reported for such climates. Cultivars of *S. miyabeana* also seem to be very well suited for SRC in the southern region because it has a greater resistance to insect and disease than *S. viminalis* and thus, shows more consistent yields (Labrecque & Teodorescu 2005).

The productivity of willows can nevertheless be negatively impacted by climate (e.g. short growing season degree days, and low precipitation and soil moisture) and soil (e.g. low nutrient availability or contaminants) (Tahvanainen & Rytkönen 1999; Trapp *et al.* 2000; Toillon *et al.* 2013). Under certain conditions, the productivity of SRC of various willow cultivars has been reported to be quite low, i.e. 1 to 5 Mg DW ha $year^{-1}$ (e.g. Heinsoo *et al.* (2009) in Estonia, Tahvanainen and Rytkönen (1999) in Finland, and (Ens *et al.* 2013) and Moukoumi *et al.* (2012) in different locations in Canada, including the dry Prairie provinces with relatively short summers). In particular, climate was shown to severely impact willow biomass production. A meta-analysis conducted in Sweden on SRC of willow with high soil nitrogen (N) contents (75-165 Kg N ha^{-1}) showed that yields

could be modeled at the landscape scale with radiation use efficiency as the main independent variable (Sannervik *et al.* 2006), whereas Price and Clancy (1986) and Guidi and Labrecque (2010) demonstrated the importance of having sufficient precipitation for optimum willow productivity. Soil nutrient availability was also shown to impact willow biomass production. Increased soil N availability following fertilization generally leads to greater willow yields, especially for soils with initially low nutrient availability (Labrecque & Teodorescu 2003). Soil pH and total calcium (CaO) were also shown to control *S. purpurea* yields of nine SRCs established from southern Ontario to central Alberta (Ens *et al.* 2013).

Willow aboveground biomass production is normally lower during the first growing season compared to subsequent years within the same rotation, whether the SRC has just been established (first rotation) or is in its second or third rotation (Verwijst 1996; Labrecque & Teodorescu 2005). However, a meta-analysis from data of 2082 willow SRCs in Sweden showed that yields increased by 60% from the first to second rotation (Mola-Yudego & Aronsson 2008). Similarly, Volk *et al.* (2011) observed an asymptotic increase of aboveground biomass from the first to the fourth rotation. This is likely explained by a delay of the rooting system to fully establish and acquire soil resources (i.e. water and nutrients). Moreover, coppicing is often done in the first stage of growth. Despite few studies on its effects on willow productivity, coppicing is reported to stimulate the production of fine roots (Berhongaray *et al.* 2015). In turn, it decreases competition by weeds and leads to resprouting of multiple and vigorous stems (Guidi *et al.* 2013). Coppicing of willow at initial stages tends to increase stem growth and maximum root diameter (Crow & Houston 2004). In this respect, producers will generally coppice after one full growing season of the first rotation to increase the number of stems per stool and to promote aboveground biomass production for following years. The benefits of coppicing on growth were reported to be small on sandy soils, probably because nutrient and water availability are intrinsically low. Positive coppicing effects were more frequently observed for clayey soils (Abrahamson *et al.* 2002). The gains on basal main stem

diameter, height and weight induced by coppicing also varied among species and clones (Guidi Nissim *et al.* 2013). As a whole, however, studies on the effects of coppicing on willow growth remain relatively anecdotic and more robust scientific studies are required to identify the conditions (and timing) under which it should be conducted.

Measuring stem diameter and height as well as total aboveground biomass after a rotation of a few years is an integrative measure of site productivity (Brown 1976; Labrecque & Teodorescu 2001). Telenius and Verwijst (1995) showed that the strong relationships between stem diameter, height and aboveground biomass allow for the development of allometric equations for the non-destructive (i.e. mostly stem diameter measurements) but diligent estimation of biomass yields of specific cultivars with satisfactory precision. This method could be used to decide whether the stands have reached financial maturity and should be harvested. Such integrative growth data, however, does not provide the detailed information needed to fully elucidate seasonal (climate, water and nutrients), coppicing and stool age (rotation associated to harvest) effects on willow growth and biomass production. For example, Mola-Yudego and Aronsson (2008) suggested that higher annualized yields from the first rotation were achieved with four growing seasons instead of five. Annual yield data could be of significant value to assess whether coppicing and number of rotations have a measurable impact on willow productivity. In this context, the objective of this study was to reconstruct annual aboveground biomass production (using a combination of stem diameters and dendrochronological measurements) in a series of willow SRCs in southern Quebec at various sites and covering different root system ages as a means to determine the impact that coppicing and rotation have had on yields.

1.3 Material and methods

1.3.1 Study sites

This study used a network of eighteen SRCs of *S. miyabeana* SX67 established at nine different sites in southern Quebec on various soil types (Tables 1 and 2). In all SRCs, SX67 cuttings of 20 to 25 cm in length were inserted in the soil to a depth of about 15-20 cm at an interval of 30 cm along a single row design with a spacing of 1.8 m between the rows (approximate density of 18,500 stools ha-1) using a planting machine. The cuttings were from one-year-old stems of about 3 m long and 1-2 cm in diameter that showed no symptom of disease on bark or wood. For the ABI, ALB, BOI, LAV, MTL, RXP, SJPJ and STR sites, row lengths of SX67 were over 100 meters at a typical monoculture site, whereas SX67 was distributed in randomized split-blocks at the HTG site (10 × 12 m), which consists of a clonal trial. Coppicing was generally done in the fall of the first growing season. However, some SRCs were either not coppiced or coppiced after the second growing season only (see Tables 1 and 2 for details). Harvesting was generally done three to five years after coppicing. Before SRC establishment, weeds were controlled using mechanical soil preparation (i.e. where stoniness was low, ploughing in the fall of the previous year was followed by cross-disking just before spring planting). Herbicides (i.e. normally glyphosate 2 to 4 L/ha) were applied before soil ploughing in the fall (Guidi *et al.* 2013). The fact that the measurements were performed in SRCs with different numbers of coppicing and rotations allowed to specifically test their effects on aboveground yields (see further details in this section).

The SRCs established at the ABI, ALB, HTG, LAV, RXP and STR sites were on former agricultural soils of varying textures, whereas MTL was planted on a sandy (loamy sand) forest soil after a mixedwood stand was harvested and the soil surface had been cleared from all residues (Table 1.2). Most sites were characterized by slightly acidic soils (pH from 5.2 to 5.6), except for soils at the BOI and HTG sites which were near neutral (pH from 7.3 to 7.5). Also, the RXP site was the only one where SRCs were established on an organic soil (Table 1.2).

Table 1.1 Mean diameter (D) and height (H) of the stems and number of stems per stool measured in the short largest rotation cultures (SRC) of SX67. Aboveground biomass yield, the years for which dendrochronolgy estimations were performed and Pearson correlation coefficient between the area of the largest diameter and aboveground biomass dry weight (r_p A:W) are reported when available.

Site	SRC	Year established	Soil sampling year	Rotation	Stem and stool ages	Field measurement year (biomass)	n	D (mm)	H (m)	Number of stems per stool	Measured yield (t ha-1)	Years that yields were estimated	rp A:W
Abitibi	ABI1	2008	2011	1	S3R4	2011	36	23.2	2.9	10.8	14.0	2009 to 2013	0.45
	ABI2	2008	2011	2	S2R6	2013	36	23.9	NA	NA	NA	2011 to 2013	NA
Albanel	ALB1	2009	2013	1	S4R5	2013	50	46.5	5.2	2.5	NA	2010 to 2013	NA
Boisbriand	BOI1	2005	2011	2	S3R7	2011	36	34.8	5.4	2.6	38.5	2009 to 2013	0.77
	BOI2	2007	NA	1	S3R5	2011‡	36	40.8	5.3	5.1	44.7	No	0.57
					S2R4	2010‡	36	32.4	4.9	5.5	31.8	No	0.20
					S1R3	2009‡	36	20.4	3.5	8.5	19.1	No	0.30
					S1R1	2007	36	11.6	1.9	3	2.9	No	0.69
Huntingdon	HTG1	2000	2012	4	S3R13	2012	36	43.7	5.5	NA	NA	2010 to 2013	NA
				3	S4R11	2009	24	50.0	6.8	5.2	NA	No	NA
					S2R9	2007	24	27.8	4.8	4.6	19.9	No	0.41
				2	S3R7	2005	24	34.0	4.5	6.5	NA	No	NA
					S2R6	2004	24	27.5	4.0	12.1	NA	No	NA
					S1R5	2003	24	16.5	2.9	11.3	15.3	No	0.35
				1	S3R4	2002	24	38.9	4.4	3	34.9	No	0.76
					S2R3	2001	24	32.4	3.7	3	21.5	No	0.81
					S1R2	2000	32	20.2	2.4	4.2	6.5	No	0.67

An ID is attributed to each SRC to distinguish them between and within sites. The column entitled Rotation and Measurement year indicate respectively the number of rotations of the SRC and the year for which growth variables were measured. The number of shrubs measured within each SRC is also indicated (n). SRCs were coppiced after one growing season, unless otherwise indicated;

† Indicates a SRC which was not coppiced;

‡ Indicates a SRC which was coppiced after two growing seasons.

Table 1.1 (Continued) Mean diameter (D) and height (H) of the stems and number of stems per stool measured in the short largest rotation cultures (SRC) of SX67. Aboveground biomass yield, the years for which dendrochronolgy estimations were performed and Pearson correlation coefficient between the area of the largest diameter and aboveground biomass dry weight (r_p A:W) are reported when available.

Site	SRC	Year established	Soil sampling year	Rotation	Stem and stool ages	Field measurement year (biomass)	n	D (mm)	H (m)	Number of stems per stool	Measured yield (t ha-1)	Years that yields were estimated	rp A:W
Laval	LAV1	2005	2011	1	S5R7	2011‡	36	44.8	6.4	2.1	46.8	2007 to 2013	0.78
	LAV2	2005	NA	1	S2R3	2007	36	30.9	4.8	5.4	23.9	No	0.60
Mont-Laurier	MTL1	2008	2011	1	S3R4	2011	36	25.3	3.2	2.8	8.4	2009 to 2013	0.79
				2	S2R6	2013	16	18.5	NA	NA	NA	2011 to 2013	NA
Roxton Pond	RXP1	2008	2011	1	S3R4	2011	36	36.3	4.6	NA	30.3	2009 to 2013	NA
	RXP2	2008	2011	2	S1R6	2013	20	15.3	NA	NA	NA	2013	NA
	RXP3	2006	No	1	S2R3	2008	36	36.3	5.1	3.1	38.7	No	0.40
	RXP4	2006	No	1	S2R2	2007†	36	23.1	3.5	9.0	15.3	No	0.29
Saint-Jean-Port-Joli	SJPJ1	2009	2011	2	S2R5	2013	50	33.0	NA	NA	NA	2009 to 2013	NA
				1	S3R3	2011†	36	35.2	4.2	2.7	27.9		0.83
	SJPJ2	2006	NA	1	S1R2	2007	36	11.5	2.0	10.9	4.3	No	0.48
					S1R1	2006†	36	6.9	1.4	2.6	1.0	No	0.18

An *ID* is attributed to each SRC to distinguish them between and within sites. The column entitled Rotation and Measurement year indicate respectively the number of rotations of the SRC and the year for which growth variables were measured. The number of shrubs measured within each SRC is also indicated (n). SRCs were coppiced after one growing season, unless otherwise indicated;

† Indicates a SRC which was not coppiced;

‡ Indicates a SRC which was coppiced after two growing seasons.

Table 1.1 (Continued) Mean diameter (D) and height (H) of the stems and number of stems per stool measured in the short largest rotation cultures (SRC) of SX67. Aboveground biomass yield, the years for which dendrochronolgy estimations were performed and Pearson correlation coefficient between the area of the largest diameter and aboveground biomass dry weight (r_p A:W) are reported when available.

Site	SRC	Year established	Soil sampling year	Rotation	Stem and stool ages	Field measurement year (biomass)	n	D (mm)	H (m)	Number of stems per stool	Measured yield (t ha-1)	Years that yields were estimated	rp A:W
Saint-Roch-de-l'Achigan	STR1	2005	2011	2	S3R7	2011	36	29.7	4.2	7.6	26.9	2009 to 2011	0.45
	STR2	2007	NA	1	S3R5	2011‡	36	32.6	4.4	3.5	41.4	No	0.71
					S2R4	2010‡	36	23.6	3.7	5.3	22.4		0.25
	STR3	2006	NA	1	S2R2	2007†	36	23.0	3.0	7.8	21.2	No	0.44
Mont-Laurier	MTL2	2006	NA	1	S3R3	2008	36	20.7	3.2	3.4	13.0	No	0.57
			NA		S2R2	2007†	36	16.1	2.5	3.1	5.4	No	0.73
Normandin	NOR1	2007	NA	1	S1R1	2007†	36	4.6	0.7	3.6	0.9	No	0.80

An ID is attributed to each SRC to distinguish them between and within sites. The column entitled Rotation and Measurement year indicate respectively the number of rotations of the SRC and the year for which growth variables were measured. The number of shrubs measured within each SRC is also indicated (n). SRCs were coppiced after one growing season, unless otherwise indicated;

† Indicates a SRC which was not coppiced;

‡ Indicates a SRC which was coppiced after two growing seasons.

The shaded area indicates SRCs that were only used to build the model presented in Figure 2.

Table 1.2 Previous land use and general climatic and soil properties of the short rotation cultures of SX67 for which maximum yield potential was estimated.

Site	Latitude	Longitude	Soil type/Previous land use	Soil preparation	First growing season‡	Annual rainfall (mm)	Annual degree-days (5°C)	Sand (% mass)	Clay (% mass)	O.M. (% mass)	pH	CEC† ($cmol_c\ kg^{-1}$)
Abitibi (ABI1 and ABI2)	48.65852	-77.635812	Clay / agriculture	Tillage	2009, 2012£	718	1609	20.6	43.1	7.9	5.6	15.4
Albanel (ALB1)	48.871624	-72.425107	Silt loam / agriculture	Tillage	2010	664	1676	32.2	9.6	10.2	6.2	18.8
Boisbriand (BOI1)	45.629525	-73.89309	Loam / hardwood forest	No till	2006, 2009	762	2368	34.9	18.9	9.2	7.5	23.7
Huntingdon (HTG1)	45.144667	-74.142097	Silty clay loam / agriculture	Tillage	2001, 2003, 2006, 2010	765	2414	18.9	27.6	11.2	7.3	38.1
Laval (LAV1)	45.553382	-73.833063	(Sandy) loam / agriculture	Tillage	2007	717	2488	49.6	11.3	5.4	5.3	6.3
Mont Laurier (MTL1)	46.455658	-75.498268	Loamy sand / mixed forest	Tillage	2009, 2012	699	2027	74.1	3.7	6.9	5.2	3.4
Roxton Pond (RXP1 and RXP2)	45.558663	-72.676412	Organic / agriculture	Tillage	2009	864	2333	46.2	5.7	67.6	5.5	68.1
Saint-Jean-Port-Joli (SJPJ1)	47.246119	-70.227056	Loam / forage	No till	2009, 2012	748	1867	30.7	22.9	9.1	5.3	12.2
Saint-Roch de l'Achigan (STR1)	45.844108	-73.632014	Sandy loam / agriculture	Tillage	2006, 2009	831	2288	59.2	8.7	4.6	5.6	7.0

‡ Multiple years indicate multiple rotations; †CEC is effective cation exchange capacity; £Only for ABI2.
O.M. refers to organic matter content measured by loss on ignition using a muffle furnace

1.3.2 Field growth survey

The SRCs were monitored non-systematically at the end of the growing season between 2000 and 2013 (Table 1.1). Each measurement was performed on sixteen to fifty shrubs in four to nine plots (5 × 5 m). Plots were selected along one or two randomly selected rows, depending on the number of plots measured. Each plot was separated by at least 20 m. The sampling in each plot consisted of four to ten healthy/vigorous successive stools along the row and on which height of the largest stem, the basal diameter at about 5 cm above the collar (later referred as largest diameter) and the number of stems per stool were measured. For each stool, all stems were then harvested for biomass measurement. Fresh weights (including the leaves) were obtained in the field using an electronic scale. Subsamples of 3 to 6 kg (integrating whole stems) were then collected, brought back to the laboratory and dried to constant weight at 70°C in an oven and reweighted to convert fresh weight to dry weight. It should be noted that sampling the stem with the largest diameter from healthy/vigorous stools was systematically applied as a means to provide a proxy for the maximum yield potential (MYP) of a site. This method was used because we were interested in reconstructing annual yields and that dendrochronological work could only be conducted on a limited number of stems per SRC. It can be considered for SX67 because studies of SX67 generally show that it produces less stems than other productive clones. For example, Guidi Nissim *et al.* (2013) reported that the number of stems per stool of SX67 did not increase significantly between the first and the third rotations, unlike *Salix eriocephala* Muhl which also has a higher number of stems per stool compared to SX67. To some extent, our approach is inspired from the methodology used in forestry to estimate site quality index for which only dominant trees (i.e. the larger stems) are selected for measurement (Carmean 1975; Perron *et al.* 2009). Using our approach could, however, be problematic for *Salix* clones that produce more stems and distribute much of the biomass in the smaller stems.

1.3.3 Soil sampling and analysis

SRC in each of the nine sites for which the field growth survey had been conducted (see Table 1.2 for specific SRCs). The samples were first air-dried in the laboratory and then sieved to pass through a 2 mm mesh. Soil pH of mineral samples were measured using a soil:water ratio of 1:2, whereas a ratio 1:10 was used for organic soil samples (i.e. RXP). Using a muffle furnace, organic C was measured by loss of ignition after 15 minutes at 575°C and then inorganic C ($CaCO_3$) after 10 minutes at 1000°C (Rabenhorst 1988). Soil particle size distribution was analyzed using the Horiba Partica LA-950v2 Laser Particle Analyzer (Horiba Instruments, Irvine, CA, USA). Samples from BOI, MTL and SJPJ were first pre-treated to destroy sesquioxides. In this respect, samples had to be bleached twice with NaOCl and thoroughly washed with distilled water. Samples from RXP contained more than 60% of organic matter and only small amounts of mineral particles. Thus, the bulk of organic matter was first destroyed by loss on ignition and then treated with NaOCl before yielding enough particles for size analysis. Soil exchangeable cations were measured by atomic absorption (Varian AA-1475, Palo Alto, US) after they were extracted using an unbuferred 0.1 M $BaCl_2$ solution (Hendershort *et al.* 2007a). Cation exchange capacity (CEC) was defined as the sum of exchangeables cations (Ca, Mg, K, Na, Mn, Al and Fe).

1.3.4 Relationship between area of the largest diameter and dry biomass

Stem and root system ages of these SRCs ranged from 1 to 7 years and from 1 to 9 years, respectively (Table 1.1). Hence, we identified the root age and the stem age in a single descriptor (i.e. SaRa where S is stem, R is root and a is age of the stem and root system). A linear relationship between the area of the largest diameter and aboveground biomass was tested for all available measurements. Between 2000 and 2013, aboveground biomass was measured twenty-four times at seventeen SRCs of the main eight sites. Two additional sites for which three more biomass samplings were available (as described above) were also included only

for this dataset as a means to build the most robust regression model. Points from this dataset are not independent considering that more than one SRC was monitored within the same site at most of the study sites. Thus, to test the site effect as a random factor, a mixed model was also performed with the area of the largest diameter as a fixed variable using *lme* function in the nlme package (R 3.01, R Core Team 2012). This function allows unbalanced experimental design. Normality of residuals of these models was validated with the Shapiro-Wilk test, whereas conditions of homoscedasticity were validated visually.

1.3.5 Estimation of basal area increment

Annual productivity of SRCs at the eight sites for which the field growth survey had been conducted and sampled for soils (see ''Soil sampling year'' column in Table 1.1 for details) was investigated using estimates of basal area increment (BAI). Root system age, numbers of coppicing and rotations varied between these SRCs. Dendrochronological analyses were therefore carried out from a set of six to twenty-two stem sections per SRC that were sampled at about 5 cm above the collar and that also exhibited the largest diameters. This sampling was completely distinct from the field growth survey described previously as it was conducted one to two years following the main survey (i.e. in 2012 and 2013, depending of site). Strain analysis (Rubino & McMarthy 2000; Johnson & Abrams 2009) was performed on the disks using a binocular coupled with WinDENDRO (Regent Instruments, Québec, Canada) after progressively sanding them with grits of 200, 400 and 600. For each stem section, a total of six rays were used to estimate the BAI of each growing season on the disk. We first calculated the total growth area corresponding to the year that the largest diameters were measured during the field survey (specific for each site). This area (i.e. sum of growth areas or BAIs) was then used as the denominator to calculate the contribution (in %) of BAI of each growing season captured on the disk, including the years following the growing season that the largest diameters were measured during the field survey. The contributions calculated on each disk were averaged by growing season for

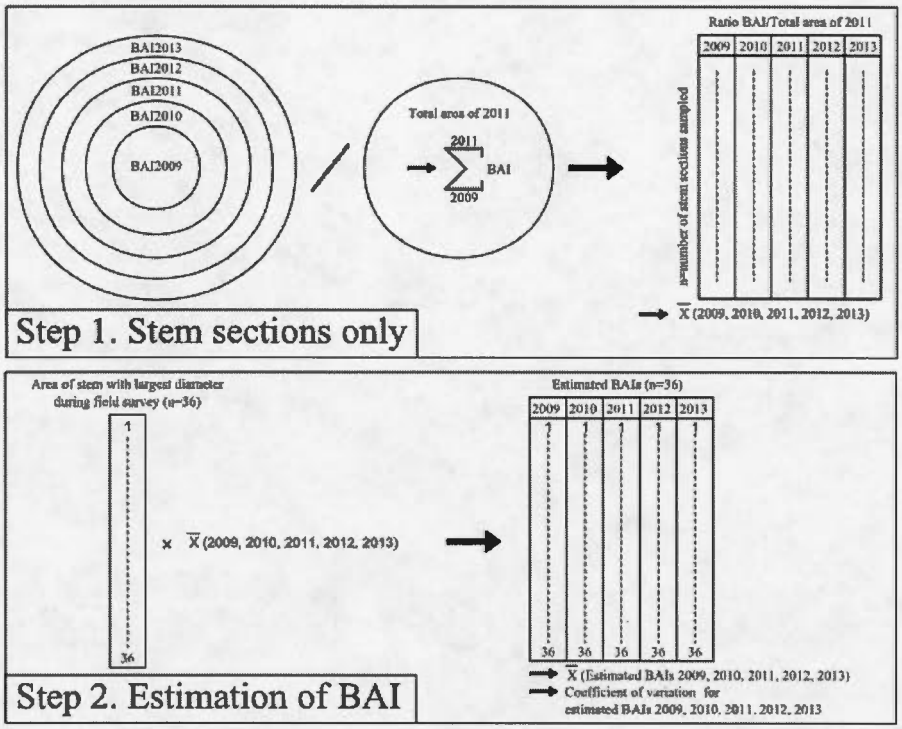

Figure 1.1 Method to estimate the basal area increments (BAI) of all growing seasons captured within the sampling of a short rotation culture of SX67

each site (Step 1, Figure 1.1). The areas of the stems with the largest diameter measured during the field survey were then multiplied by the average contribution of BAI of each growing season captured at the SRC as an estimation of BAI for each respective growing season. These estimates of BAI calculated on each disk were finally averaged by growing season for each site (Step 2, Figure 1.1).

To validate our approach, stems with the largest basal diameters that were measured in the field in 2011 at six SRCs (i.e. ABI1, BOI1, HTG1, MTL1, LAV1 and RXP1) and other stems were again measured in 2013 in the same SRCs. The area of largest diameters measured in 2011 (9 plots × 4 stools per plot = 36 stools) were statistically compared to the 2011 estimates (5 plots × 10 stool per plot = 50 stools) obtained based on hindcasts from the stems with the largest basal diameters measured in 2013. To compare the estimated and measured values, a mixed model was developed with site as a random effect and estimated vs. measured as a fixed factor using the function *lme* of the nlme package (R 3.01, R Core Team 2012). Because the condition of normality of residuals was not met

with the raw data (tested with the Shapiro-Wilk test), the mixed model was performed with log-transformed data.

1.3.6 Estimation of annual aboveground biomass production and maximum potential

The equation of the linear relationship between area of the largest diameter and aboveground biomass (Figure 1.2) was used to hindcast annual aboveground biomass yields for the nine selected SRCs. Independence of data points was accepted based on the non-significant site effect (random factor) in the mixed model and the fact that the slopes of both models were roughly similar (see Results section). Thus, the model was used to convert the estimated BAI for each growing season to annual dry mass yields per shrub. It was then multiplied by 18,500 (i.e. the number of seedlings planted per hectare) to estimate the annual yields. The estimated annual yields are clearly overestimates because mortality was not taken into account, but as indicated earlier, our numbers are proposed as an estimate of MYP.

At four of the sites, it was also possible to estimate annual aboveground biomass production of subsequent rotations using the same dendrochronological approach and linear model described above. At the RXP site, RXP2 (next to RXP1 and with root systems of the same age) was harvested late in the fall of 2012. We thus measured the basal diameter of the largest stem of twenty shrubs in the fall of 2013 (i.e., first growing season of the second rotation) and calculated the BAI. Also, ABI2 (next to ABI1 and with root systems of the same age) was harvested just after snow thaw in the early spring of 2012 and the basal diameter of the largest stem of twenty shrubs with two growing seasons was measured at the end of the 2013 growing season. Producers harvested SJPJ1 at the end of the 2011 growing season so that the largest basal diameter of fifty stems (5 plots × 10 stools per plot) with two growing seasons were measured again at the end of the 2013 growing season. Finally, at MTL1, we harvested SX67 for biomass

measurement at the end of the 2011 growing season. Contiguous shrubs of sampled plots were also cut to facilitate handling. The same stools therefore regrew without light limitation due to an overall low height (not shown) and stem density of neighbor shrubs. The basal diameter of the largest stem of twenty shrubs was again measured on stems with two growing seasons. At ABI2, MTL1 and SJPJ1, stem sections were sampled to reconstruct the BAI of the first and second year of growth as explained above. The shrubs measured at this site were therefore assumed to be representative of MYP.

The fact that stem and root system ages were different between sites necessitated that we identified a temporal threshold for MYP for comparison, i.e. the year that the MYP was reached. On the one hand, for SRCs with only one rotation, the MYP threshold was identified as the first growing season for which the yield was not lower from those of subsequent growing seasons. This was done by determining whether the upper limit of the range encompassed by the coefficient of variation of the estimated BAI (step 2, Figure 1.1) of the second growing season reached or exceeded the mean of one of the following growing season. If the second growing season did not fulfil this condition, then the procedure was repeated with the third growing season. At that point, the test was conclusive at all SRCs (i.e. MYP was reached in the third growing season or less). On the other hand, for SRCs in their second rotation or more, the MYP threshold was identified as the second year of growth of the current rotation because the first growing season following establishment or harvest is generally characterized by the lowest aboveground biomass yields, due to a high initial C demand of resprouting stems which is, in large part, satisfied by root reserves (von Fircks *et al.* 2001). Based on the assumption that annual productivity increases asymptotically (Kopp *et al.* 2001) until MYP is reached, the problem of comparing annual productivities between one SRC for each of the eight sites is overcome by using yields that appeared to be no longer considerably limited by root development. Because the coppicing year varied between SRCs (after one or two growing seasons) and that some SRCs were not coppiced at all, the year that MYP was reached is later

reported in the manuscript as the total number of years since willow establishment.

1.3.7 Data analysis

Mixed models were developed to explain the area of the largest diameter-to-height ratios with site as a random factor and stem age as a fixed factor. Stem age was considered as a quantitatively continuous variable as well as a binary variable, allowing the comparison between the area of the largest diameter-to-height ratios of stems with one growing season and stems with more than one growing season. Normality of residuals was tested by the Shapiro-Wilk test using the function *shapiro.test*. The R^2 of the models were obtained by squaring the r Pearson coefficients of the correlation between fitted values and raw data. Also, a linear regression was developed using the number of stems per stool as the response variable and the age of the root system as the explanatory variable.

Using one-way ANOVA, we compared growth variables (i.e. basal diameter of largest stem height and aboveground dry biomass) between shrubs of the same stem age but of different stool (or root system) ages. Because the condition of normality was not systematically fulfilled, comparisons were made by ANOVA using a permutational test to overcome the deviations in the normality of the data (Legendre 2007). This assumes that ANOVA is quite robust against relative non homoscedasticity. Data were always grouped and tested by site. In several cases, the estimated basal diameters of the largest stems were compared to measured basal diameters of the largest stems from another SRC of the same site or from the same SRC but for a different rotation. This test systematically served to assess the age effect of the rooting system on growth within a site (later referred to as the stool age effect). By doing so, the effects of coppicing and harvesting were also tested. However, this test was not systematically possible at each site.

In order to test for the effects of stem and stool (or root system) ages on inter-annual variations in BAI, one-tail paired t-tests (paired by site and rotation) were

performed on the reconstructed annual growth data. We tested whether BAI after one growing season differed from BAI after two seasons, BAI after two growing seasons differed from BAI after three seasons, and BAI after three growing seasons differed from BAI after four seasons. This was done without considering whether MYP was reached or not. To statically compare annual aboveground biomass yields between sites, estimated yield data had to be produced for each plot within a site (n = 9). Therefore, the average stem diameter area of all the plots in a SRC was used as the denominator to calculate the difference (in %) with the average stem diameter area of each plot. To compute values that reflect MYP, only annual yields of growing seasons that had reached MYP were used to calculate an average annual yield for the SRC. This average annual yield was then multiplied by the percent differences between average stem diameters (i.e. plot vs SRC) to obtain a single annual biomass yield value for each plot of each SRC that reflects MYP. By doing so, it is recognized that the time to reach maximum yield varies depending on the conditions that prevail at a site. It is also representative of soil nutrient availability and global variations of hydroclimatic conditions that impact growth at a site. One-way ANOVA with permutational test was also performed to compare MYP at plot scale between sites.

To compare our ability to estimate aboveground biomass yield using the area of the largest basal diameter as well as other growth variables such as height, root age, stem age, number of rotation and number of stems per stool, we partitioned the variance in biomass as proposed by (Peres-Neto *et al.* 2006). This allowed to assess: (1) how much of the variance in biomass yield explained by the area of the largest basal diameter is concomitantly explained by another growth variable and (2) if that other growth variable could explain a part of the variance in biomass yield that is not explained by the area of largest basal diameter. Partitioning was done using the function varpart available in the *vegan* package. Variances that were explained only by the area of the largest basal diameter or only by another growth variables were tested by permutation through partial canonical redundancy

analysis. The function rda available in the *vegan* package was used. All statistics described above were done using R 3.01 (R Core Team 2012).

1.4 Results

1.4.1 Aboveground biomass yields and maximum potential

A significant relationship was found between the area of the largest stem and aboveground dry biomass (Figure 1.2). The intercept of this model was near zero. The linear relationship between the area of the largest stem and dry biomass was also significant with the mixed model ($p < 0.001$, $n = 28$, results not shown), but site effect (random factor) was not significant ($p = 0.38$). The slope of the linear regression (0.001725) was also similar to that of the mixed model (0.001718). The independence of measurements was therefore assumed and the linear equation in Figure 1.2 was used to estimate MYP as explained in the Material and methods section. Another series of mixed models did not reveal a significant difference between the area of the largest stems measured in 2011 and estimates of area of the largest stems for that same year ($p = 0.43$, $n = 516$, results not shown), thus confirming the validity of our method to hindcast aboveground biomass. The significant site effect (random factor) confirms that yields differed between sites.

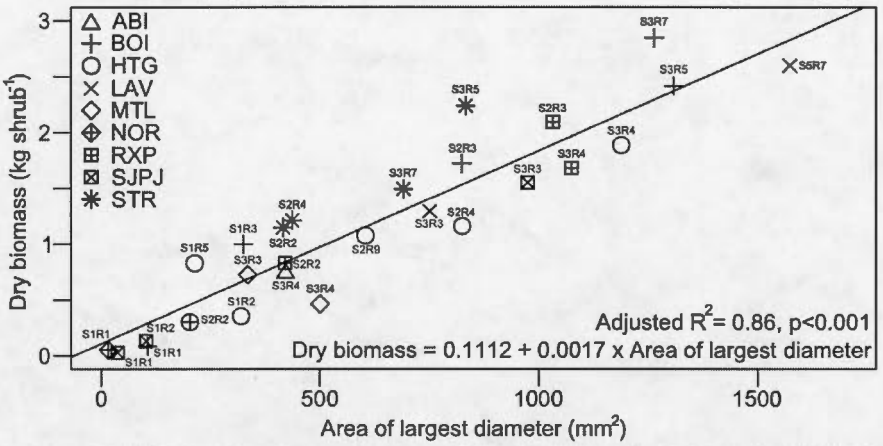

Figure 1.2 Linear regression model between the diameter measured on the largest stem and dry aboveground biomass of shrubs. Each data point refers to an average of 24 to 36 shrubs. A different symbol is attributed for each site. Data labels indicate the number of growing seasons of the stems (S) and roots (R). For example, the label S1R5 means a one year-old stem and a five year-old root system.

Figure 1.3 illustrates growth dynamics of six SRCs that have completed only one rotation, i.e at least four years. The MYP at MTL1 was reached in the second growing season following coppicing (i.e. S2R3), whereas MYP was reached at ABI1 and RXP1 in the third growing season following coppicing (i.e. S3R4). Coppicing was not done at SJPJ1, but MYP was also reached in the third growing season (i.e. S3R3). At LAV1, MYP was reached in the second growing season (i.e. S2R4). For this SRC, because coppicing was performed after the second growing season, it is not possible to know if MYP could have been reached in a shorter time. Also, annual yields did not decrease after seven years of growth at that site (i.e. S7R9). At least two full rotations were completed at BOI1, HTG1 and STR1. The MYP for these SRCs was reached in the second growing season following coppicing (i.e. S2R6, S2R12 and S2R6, respectively, Figure 1.4).

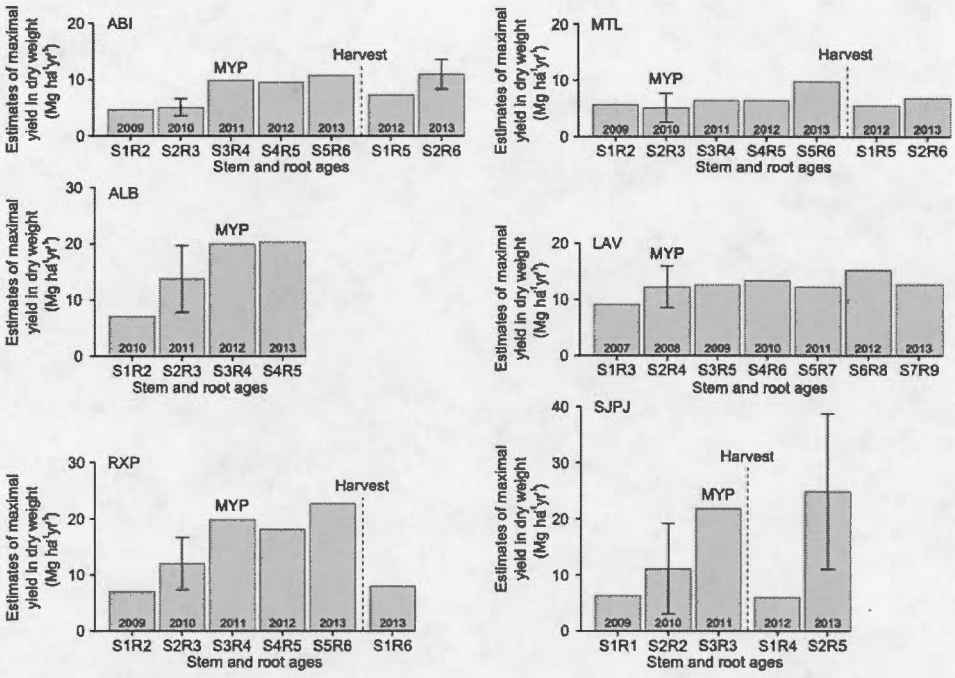

Figure 1.3 Estimated annual aboveground biomass yields of the six short rotation cultures of SX67 that have completed only one rotation (i.e. at least four years). The values correspond to slight overestimations of actual yields because mortality was not taken into account. The root age and the stem age are identified in a single descriptor (i.e. SaRa where S is stem, R is root and a is age of the stem and root system). Error bars (standard deviation) were centered on the second growing season to illustrate the year that maximum yield potential (MYP) was reached, which is indicated over the bar of the appropriate year.

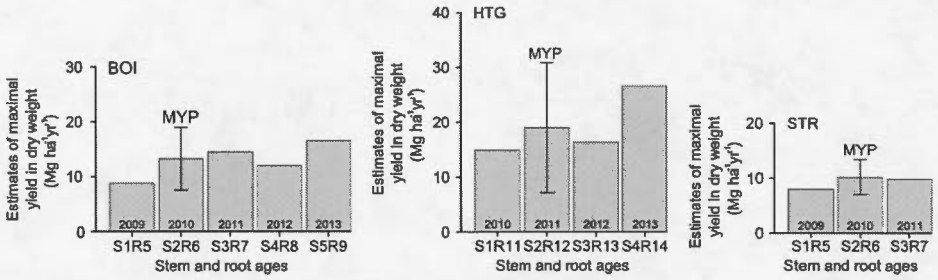

Figure 1.4 Estimated annual aboveground biomass yields of the last rotation in three short rotation cultures of willow that have completed at least two rotations. The values correspond to slight overestimations of actual yields because mortality was not taken into account. The root age and the stem age are identified in a single descriptor (i.e. SaRa where S is stem, R is root and a is age of the stem and root system). Error bars (standard deviation) were centered on the growing season for which the diameter of the largest stem was measured. The year that maximum yield potential (MYP) was reached is indicated over the bar of the appropriate year.

On average, MYP estimates at MTL1, STR1 and ABI1 were significantly lower than all other sites (i.e. 7.0, 10.1 and 10.4 t ha^{-1} $year^{-1}$, respectively), whereas SJPJ1and HTG1 had the highest MYP estimates (i.e. respectively 23.8 and 21.1 t ha^{-1} $year^{-1}$) (Figure 1.5). The MYP estimates at ALB1 and RXP1 (i.e. respectively 20.5 and 18.5 t ha^{-1} $year^{-1}$) were significantly higher than those at BOI1 and LAV1 (i.e. 14.3 and 13.2 t ha^{-1} $year^{-1}$, respectively, Figure 1.5).

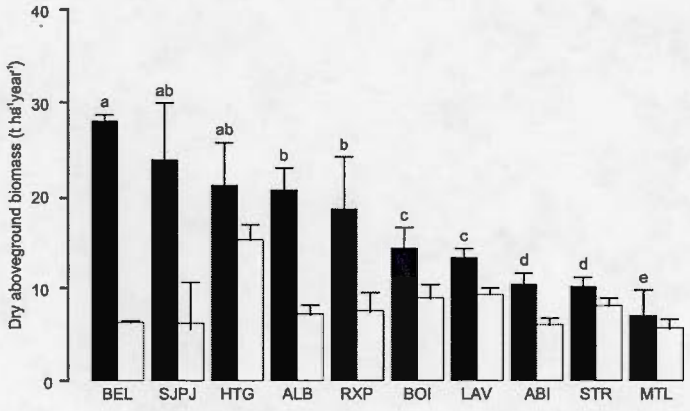

Figure 1.5 Mean maximum yield potential (MYP, black bars) and annual aboveground biomass yield of the first growing season (white bars) for each site. In the case where there is no significant difference in MYP between sites (permutational ANOVA), the same letter is assigned.

Basal area increments were significantly lower after one growing season compared to the second growing season when grouped and compared by site (model 1, Table 1.3), but no significant effect was found when comparing the second and third growing seasons, or comparing the third and fourth growing seasons (respectively models 2 and 3, Table 1.3).

Table 1.3 Results of statistical differences in basal area increment between growing seasons (GS).

Model	Tested groups	P value
1	GS1 < GS2 (n = 26)	0.002
2	GS2 < GS3 (n = 14)	0.361
3	GS3 < GS4 (n = 14)	0.201

1.4.2 Partitioning of the variance in aboveground biomass biomass

Partitioning of the variance showed that the area of the largest diameter was the most robust proxy to explain aboveground biomass (Table 1.4). Height shared 0.81 of the adjusted R^2 with the area of the largest diameter, and it added 0.03 ($p < 0.05$) to the adjusted R^2, meaning that 3% of the variance was not explained by the effect of the area of the largest diameter. The highest adjusted R^2 (0.90) was obtained by combining the number of rotations to the area of the largest diameter. The adjusted R^2 was not improved by combining either root age, stem age or the number of stems per stool to the area of the largest diameter.

Table 1.4 Partitioning of the variance of dry aboveground biomass between the area of the largest diameter and a second explanatory growth variable (i.e. height, root age, stem age, rotation number or number of stems per stool).

Model	n	Adj. R^2 of the area of largest diameter ℂ second explanatory variable	Adj. R^2 of the area of largest diameter ∩ second explanatory variable	Adj. R^2 of the second explanatory variable ℂ Area of largest diameter	Adj. R^2
1	27	0.05**	Height		0.89
			0.81	0.03*	
2	27	0.49**	Root age		0.86
			0.38	0.00	
3	27	0.37**	Stem age		0.86
			0.49	0.00	
4	27	0.73**	Rotation number		0.90
			0.13	0.04*	
5	26	0.86**	Shoot number		0.86
			0.00	0.00	

All linear relationships are positive. Models are significant at α <0.05(*) and <0.01(**). The column entitled Adj. R^2 of the area of largest diameter ℂ second explanatory variable indicates the adjusted R^2 of the variance explained by the area of the largest diameter which does not overlap the effect of the second explanatory growth variable. The column entitled Adj. R^2 of the area of largest diameter ∩ second explanatory variable indicates the adjusted R^2 of the variance explained by both the area of the largest diameter and the second explanatory growth variable. The column entitled Adj. R^2 of the second explanatory variable ℂ Area of largest diameter indicates the adjusted R^2 of the variance explained by the second explanatory growth variable which does not overlap the effect of the area of the largest diameter. The column entitled Adj. R^2 indicates the adjusted R^2 of the variance explained by the cumulative effect of the area of the largest diameter and the second explanatory growth variable.

1.4.3 Relationships between growth variables and stem/root ages

Mixed model analysis showed that area of the largest diameter-to-height ratio significantly increased with the number of growing seasons, whereas site effect (random effect) was not significant (model 1, Table 1.5). This ratio changed most dramatically from the first growing season to the second growing season. The major part of the variance could also be explained with a binary variable representing the first and subsequent growing seasons (model 2, Table 1.5). Site effect was significant in this second model. This means that most of the variation

in area of the largest diameter-to-height ratios explained by the mixed models is due to changes occurring during the first two growing seasons.

Table 1.5 Results of mixed model analysis of the area of the largest diameter-to-height ratios as the response variable, site effect as the random factor and stem age as the fixed variable. Model 1 uses stem age as a continuous variable, whereas model 2 uses it as a binary variable (model 2). Model 3 is a linear regression with the number of stems per stool as the response variable and the age of the root system as the explanatory

Model	n	Response variable	Random factor	Fixed variables	R^2
1	26	Area of largest diameter-to-height ratio	Site NS	Stem age***	0.79
2	26	Area of largest diameter-to-height ratio	Site**	1st growing season of a harvesting cycle***	0.61
3	6†	Number of stems per stool	No	Age of root system**	0.54

Models are significant at α <0.01(**) and <0.001(***).
†1st growing season of a harvesting cycle.

1.4.4 Growth differences following coppicing

Based on data from SJPJ2 and BOI2, coppicing performed respectively after one and two growing seasons resulted in large increases in the area of the largest diameter and aboveground biomass (Table 1.6). At the RXP site, a comparison between three SRCs suggests that coppicing increased the area of the largest diameter and aboveground biomass yield after two growing seasons within the first rotation. Coppicing performed after the second growing season at the STR site had no effect on area of the largest diameter or biomass yields.

Table 1.6 Results of statistical differences of the area of the largest stems (A) and aboveground biomass (Yield) measured before and after the stools were coppiced (first rotation only). Differences in aboveground biomass yield of those stools are also reported. The root age and the stem age were identified in a single descriptor (i.e. SaRa where S is ste, R is root and a is age of the stem and root system).

SRC(s)	Year of coppicing	n	Stem and stool ages	A	Yield
BOI2	2008	72	S1R1 vs S1R3	+ 192%***	+ 734%***
SJPJ2	2006	72	S1R1 vs S1R2	+ 153%***	+ 691%***
RXP4 vs. RXP1	Not performed vs 2008	72	S2R2 vs S2R3	+ 23%**	NA
RXP4 vs. RXP3	Not performed vs 2006	72	S2R2 vs S2R3	+ 148%***	+ 151%***
STR3 vs. STR2	Not performed vs 2008	72	S2R2 vs S2R4	+ 5% NS	+ 6% NS

Differences (coppicing effect) before and after coppicing were tested by ANOVA with permutational test. Data were always grouped and tested by site or by short rotation culture (SRC). The age of the stems that were compared is indicated in the column Stem age. Models are significant at α <0.01(**) and <0.001(***). An ID is attributed to each SRC to distinguish them between and within sites. The shaded area indicates that the effect of coppicing was tested using the same plots within a SRC, whereas the unshaded area indicates that the effect was tested between different SRCs within a site. At BOI2 and STR2, coppicing was done after two growing seasons, whereas it was done after one year at RXP1, RXP3 and SJPJ2. The number of stools measured is indicated (n).

1.4.5 Growth differences between rotations and stool ages

At the ABI site, a clear rotation effect was observed. Area of the largest diameter at the end of the first and second growing seasons respectively increased by 87% and 130% between the first and second rotations (i.e. S1R2 vs S1R5, and S2R3 vs S2R6, Table 1.7). At SJPJ1 where coppice was not done, area of the largest diameter at the end of the first growing season did not change significantly between the first and second rotations, but area of the largest diameter at the end of the second growing season (the coppice year) increased by 66% between the first and second rotations (i.e. S1R1 vs S1R, and S2R2 vs S2R5, Table 1.7). In contrast, at both RXP1 and MTL1, shifting from the first rotation to the second rotation did not significantly increase the area of the largest diameter at the end of the first growing season (i.e. S1R2 vs S1R5), and at MTL1 after the second growing season (i.e. S2R6, Table 1.7). A special case to study the effects of rotation on biomass yields is HTG1 because four full rotations are accounted for. No specific trend was observed at HTG1 in terms of rotation effect. Even if area

of the largest diameter of the first rotation was lower than the second rotation, the number of stems per stool in the second rotation was higher (results not shown), which explains the higher yield observed after the first growing season of the second rotation compare to the first rotation (i.e S1R2 vs S1R5, Table 1.7). The third rotation was likely among the least productive. After four rotations at the HGT site, yields remained very high (Figure 1.4).

At the BOI site, at the end of the first growing season, the area of the largest diameter was 38% higher on three year-old stools than on five year-old stools (Table 1.7). However, this difference decreased over time to 34% at the end of the second growing season and to 26% at the end of the third growing season (Table 1.7). Similarly, differences of area of the largest diameter at HTG1 decreased over the growing seasons when comparing the four rotations. Whether the differences were positive (i.e. first vs. third rotations, first vs. fourth, second vs. third and third vs. fourth) or negative (i.e. first vs. second rotations), the differences were: (1) much larger when comparing the first year of growth to other growth years, and (2) dramatically lower when only older stems were compared. At SJPJ1, however, no significant difference was found at the end of the first growing season, although it was strongly significant at the end of the second growing season, probably because MYP was already reached in the second rotation. Also, at STR site, while no significant difference was found at the end of the second growing seasons between the first and second rotations, area of the largest diameter and biomass yield at the end of three growing seasons decreased significantly (Table 1.7).

Table 1.7 Results of statistical differences of the area of the largest stems (A) measured from stools of the same ages but of different rotations (i.e. different stool ages). Differences in aboveground biomass yield of those stools are also reported. The root age and the stem age were identified in a single descriptor (i.e. SaRa where S is stem, R is root and a is age of the stem and root system).

SRC(s)	Rotation	n	Stem and stool ages	A	Yield
HTG1	1 vs 2	56	S1R2 vs S1R5	- 33%**	+ 134%***
		48	S2R3 vs S2R6	- 29%$^{\pounds}$	NA
		48	S3R4 vs S3R7	- 23%$^{\pounds}$	NA
	1 vs 3	48	S2R3 vs S2R9	- 28%*	-7% NS
	1 vs 4	60	S1R2 vs S1R12	+ 37.4 %*	NA
		60	S2R3 vs S2R13	+ 23% NS	NA
		60	S3R4 vs S3R14	+ 29% NS	NA
	2 vs 3	60	S2R4 vs S2R9	+ 2% NS	NA
	2 vs 4	60	S1R5 vs S1R12	+ 107%***	NA
		60	S2R6 vs S2R13	+ 75%**	NA
		60	S3R7 vs S3R14	+ 68%**	NA
	3 vs 4	60	S2R9 vs S2R13	+ 71%**	NA
		74	S4R11 vs S4R15	+ 9%$^{\pounds}$	NA
MTL1	1 vs 2	52	S1R2 vs S1R5	- 2% NS	NA
		52	S3R3 vs S2R6	+ 2% NS	NA
RXP1	1 vs 2	56	S1R2 vs S1R6	- 4% NS	NA
SJPJ1	1 vs 2	86	S1R1 vs S1R4	- 6% NS	NA
		86	S2R2 vs S2R5	+ 66%***	NA
ABI2 vs. ABI1	1 vs 2	56	S1R2 vs S1R5	+ 87%***	NA
		56	S2R3 vs S2R6	+ 130%***	NA
BOI2 vs. BOI1	1 vs 2	72	S1R3 vs S1R5	- 38%***	NA
		72	S2R4 vs S2R6	-34%***	NA
		72	S3R5 vs S3R7	- 26%**	- 14% NS
STR2 vs. STR1	1 vs 2	72	S2R4 vs S2R6	+ 5% NS	NA
		72	S3R5 vs S3R7	- 16%*	- 31%**

Differences (rotation or harvesting effect) between stools of the same ages but of different rotations were tested by ANOVA with permutational test. Data were always grouped and tested by site or by short rotation culture (SRC). Models are significant at α <0.1($^{\pounds}$), α <0.05(*), α <0.01(**) and <0.001(***). An ID is attributed to each SRC to distinguish them between and within sites. The shaded area indicates that the effect of rotation was tested using the same plots within a SRC, whereas the unshaded area indicates that the effect was tested between different SRCs within a site. The column Rotation indicates the rotations that were compared with ANOVA. The number of stools measured is indicated (n).

1.5 Discussion

1.5.1 Estimation of aboveground biomass yields

Several non-destructive allometric models to estimate *Salix* spp. aboveground biomass using various measurements of living stems as input variables have been developed (Heinsoo *et al.* 2002; Nordh & Verwijst 2004; Arevalo *et al.* 2007; Ens *et al.* 2009; Hangs *et al.* 2011). The positive relationship between stem diameter and aboveground biomass is normally expressed by an exponential function (Tahvanainen & Rytkönen 1999; Amichev *et al.* 2011). The predictive power of these models is generally greater (i.e. up to $R^2 = 0.99$) than that of the model used in this study to calculate annual aboveground biomass yields. The difference is likely due to the fact that we considered only the main stem from each stool in our calculations, whereas most other models integrate all stems from a given stool. At HTG1, for example, the sum of basal areas of all living stems from each stool (M. Labrecque, unpublished data) was strongly related to aboveground biomass ($R^2 = 0.85$, data not shown), whereas the area of the largest diameter was more weakly related to biomass ($R^2 = 0.45$, data not shown). It should be noted that modeling aboveground biomass yield with the area of the largest diameter instead of the largest diameter alone has linearized the relationship, most likely because area is more representative of biomass accumulation than diameter (Pedersen 1998). Similarly, allometric models to estimate aboveground biomass are often based on linearized diameter with log transformation, second order polynomial of diameter or the area of cross sections (Verwijst & Nordh 1992; Verwijst 1996; Heinsoo *et al.* 2002; Arevalo *et al.* 2007).

Pearson correlation coefficients between the area of the largest diameter and aboveground biomass yield varied strongly within each site (Table 1.1). Biomass yield was correlated with the area of the largest diameter, but several coefficients were low. Again at HTG1, for example, the coefficient of correlation was 0.67 using data of the first growing season and rotation following coppicing, whereas it was 0.35 using data of the first growing season but of the second rotation, which was characterized by a high number of stems per stool (Table 1.1). To obtain a

high fit between stem diameter and aboveground biomass, Sevel *et al.* (2012) argued that a model must be site-specific and it must ideally be developed from yearly data to fully account for carbon allocation in aboveground components. Conversely, Arevalo *et al.* (2007) reported that, despite aboveground biomass being strongly influenced by site and climatic conditions, relatively robust regression models between stem diameter and biomass can be developed at the landscape scale. These authors stated that even if some accuracy is lost, the approach of measuring stem diameter only overcomes the limitation imposed by the energy (time and effort) and cost required to build allometric models specific to the sites and years. Our results corroborate this latter conclusion. This is likely because area of the largest diameter is the consequence of maximum growth of all stems of the stool, while it is also a large part of aboveground biomass. The use of the general linear function (Figure 1.2) to estimate aboveground biomass production is therefore rationalized for three main reasons: (1) it has a high R^2, (2) its intercept falls very close to the origin, and (3) the large differences in measured total biomass (after three growing seasons) between sites and the non-significant site effect when it is considered as a random factor warrant that the model is suitable to estimate and compare annual aboveground biomass production between sites.

An additional part of the residuals in aboveground biomass was explained by the height and the number of rotations (i.e. 3% and 4%, respectively), but the area of the largest diameter remained the variable which best explained the variance in biomass (Table 1.4). Across sites, the variance in aboveground biomass was not explained by the number of stems per stool, probably because the biomass of SX67 is allocated in a few large stems and especially in the largest stem (M. Fontana, personal observation). For the first growing season, the number of stems per stool was significantly explained by the age of the root system (Table 1.5), but all the variance in aboveground biomass explained by root age was captured by the area of the largest diameter (Table 1.4). The residuals of the models in Table 1.4 likely mean that some factors influencing biomass yields and specific to site,

growing season and wood quality (e.g. density) were not captured from our measurements. For example, HTG1 was the only case for which the area of the largest diameter of the first growing season decreased (i.e. between the first and second rotation), whereas aboveground biomass increased (i.e. S1R2 vs S1R5, Table 1.7). This was due to a concomitant increase in height and number of stems per stool by 21% ($p < 0.01$) and 270% ($p < 0.001$), respectively (results not shown). As a whole, however, our approach of using the area of the largest diameter to estimate aboveground biomass holds quite well for SX67 across the network, as indicated by the partitioning of the variance in biomass.

Soil and climatic variation encompassed by our SRC network (Table 1.2) clearly had a large impact on aboveground biomass yields (Figures 1.3 to 1.5). The range of our MYP estimates (i.e. from 7 to 23.8 t ha^{-1} $year^{-1}$, Figure 1.5) roughly covers values reported in the literature for SRC of *Salix spp.* under temperate climates. For example, a similar range of biomass yields was reported for *S. viminalis* in northern Europe, i.e. from 10 t ha^{-1} yr^{-1} (Tahvanainen & Rytkönen 1999) to 25 t ha^{-1} yr^{-1} (Szczukowski *et al.* 2002). In Canada, productivity of *S. miyabeana* was reported as dramatically low (i.e. 1.2 t ha^{-1} yr^{-1} for a rotation of four years) in the dry Canadian Prairies (Moukoumi *et al.* 2012) or as very high (i.e. beyond 25 t ha^{-1} $year^{-1}$) on loamy clay soils under a wetter and relatively warmer climate in southern Quebec (Guidi Nissim *et al.* 2013).

At HTG1, by the time it was in its fourth rotation, the stools had merged and thus, the shrubs could not be easily differentiated in the field. The number of stems per surface area was not monitored, but it appeared similar to the other sites. However, since a stool density of 18,500 per ha was used to estimate biomass yields, it is likely that the bias (i.e. systematic overestimation) is larger for HTG1 than for the other sites. Similarly at MTL1, the shrub density was strongly overestimated because a significant ground competition resulted in strong mortality within micro-sites (for which measurements were not performed). Thus, we believe that the yield estimate for this SRC is representative only for healthy shrubs without competition. A rapid visual assessment of mortality led to the

conclusion that mortality was low for all other sites. Comparatively, the mortality of SRCs of *S. viminalis* and *S. discolor* in southern Quebec has been estimated at less than 10% at the end of first rotation, with about 20,000 cuttings per hectare (Labrecque & Teodorescu 2003).

1.5.2 Effect of coppicing on growth and yield

After the first or second growing seasons following willow establishment, it is customary to coppice. In this study, the effects of coppicing have been tested for the first growing season at BOI2 and SJPJ2 and for the second growing season at the RXP and STR sites (Table 1.6). The benefits of coppicing were obvious at sites where soil CEC was not dramatically low (i.e. the organic soil at the RXP site or the mineral soil with significant silt and clay at BOI2 and SJPJ2), favoring major increases in the area of the largest diameter and aboveground biomass yield. Conversely, no gain from coppicing was detected at the STR site as the soil is characterized by a low CEC (sandy loam) that supports low yields. We could not directly validate our inference on the influence of coppicing for the other SRCs supported by sandy soils, i.e. MTL and LAV, because comparison of the area of the largest stems and of the aboveground biomass yields before and after coppicing was not possible. However, the SRCs at the LAV site allowed for an indirect assessment of the effect of coppicing on stem diameter. Both LAV2 and LAV1 were established in 2005, but coppicing was performed after one and two growing seasons, respectively. The areas of two year-old stems with the largest diameters at LAV2 (in 2007, S2R3) were 17% higher ($P < 0.05$, results not shown) than the areas of the largest diameters of two-year old stems at LAV1 (in 2008, S2R4). This is likely because the growing conditions of the two years following coppicing at LAV2, i.e. 2006 and 2007, favored slightly higher aboveground biomass yields compared to those following coppicing at LAV1, i.e. 2007 and 2008. Because the stems with two growing seasons were measured at LAV2 on stools that were three years and that stems of the same age at LAV1

were measured on stools that were four years, the slightly larger two-year old stems at LAV2 compared to LAV1 suggests that the growth of the largest stem was not limited at LAV2. In turn, MYP at LAV2 could also be reached during the third growing season after establishment. At the LAV site, we speculate that root development did not limit aboveground productivity after two growing seasons as we have no data on roots to support this.

Our findings therefore corroborate previous studies showing a beneficial effect of coppicing on yields of willow shrubs with a young root system when it is supported by a soil that has a relatively high CEC (e.g. clay to loam or organic as exemplified by the RXP site) and that only small effects of coppicing on yields are expected in the case of soils with coarse texture (e.g. sand) (Abrahamson *et al.* 2002). Crow and Houston (2004) showed that the development of the root system is highly influenced by harvesting, suggesting that coppicing concomitantly limits the maximum root and stem diameters. They tested the rotation length of the harvest and did not report any significant difference in the number of fine roots (i.e. < 2 mm). Based on our data, it is possible that the yield benefits of extending the length of the first rotation by one year (i.e. four years without any coppice) are larger than introducing coppicing and shortening the length of the rotation by one year (i.e. the coppice year combined with the following 3 years). However, it is impossible to fully assess if there were any benefits based on our data.

1.5.3 Effect of stem age across rotations

Within one rotation, our results suggest a significant increase in BAI only between the first and second growing seasons, independently of the number of rotations, but not between the second and the third, nor between the third and the fourth growing seasons (models 1, 2 and 3, Table 1.3). Such observations were also made in other SRCs within the first rotation (Christersson 1986; Szczukowski *et al.* 2002). A high bark-to-wood ratio for small diameter stems (i.e. under 20 mm) suggests a mass-relative high nutrient requirement in aboveground biomass

during the first growing season compared to the following growing seasons (Adler *et al.* 2005). Furthermore, resprouting of willow stems suggests a strong demand on carbon reserves until stems contain enough mature leaves to reach energy independence – starch reserves in roots are used after coppicing for initial stem growth (Von Fircks & Sennerby-Forsse 1998). Therefore, the low productivity of SX67 during the first growing season appears to be a physiological trait that other willow cultivars possess.

At HTG1 and BOI1, the differences in the area of the largest diameter between stems of the same age (i.e. comparing the first rotation to the second rotation) have systematically decreased with stem age (years 1, 2 and 3, Table 1.7). The growth difference between stems with one growing season (i.e. between the first two rotations) was particularly high. Carbon allocation was probably more evenly balanced between the stems within the first growing season after coppicing, while some stems began to dominate and others died out in the following growing seasons (Verwijst 1991). Consequently, the area of the largest diameter-to-height ratio decreased dramatically between the first and subsequent growing seasons (model 2, Table 1.3). This response was also site-specific. The increase in aboveground biomass in the first growing season compared to the following ones in the first rotation was larger than the increase in the first growing season compared to the following ones in the second rotation (Figures 1.3 and 1.4). However, shade is dramatically decreased after coppicing and in turn, strong weed regrowth can increase competition for resources (light, water and nutrients) (Sage 1999). Additionnally, root:shoot ratio can suddenly increase following coppicing, which require a strong carbon cost for root respiration compared to subsequent growing seasons (Salomón *et al.* 2013). Total yield was, however, generally higher during the second rotation (see section below) because, in part, the productivity after the first growing season in the second rotation was greater than that of the first rotation. This also suggests that the productivity of the first growing season of the first rotation is not optimal because of an under-developed rooting system.

1.5.4 Delays in reaching maximum yields

Kopp *et al.* (2001) reported that coppicing stems for ten consecutive years resulted in asymptotic increases in aboveground biomass and reached maximum values after two to three growing seasons in fertilized plots and after four to five growing seasons in non-fertilized plots. A similar lag effect to reach MYP was also apparent for our sites. Our study generally highlights that MYP could be reached after two to three growing seasons after SX67 was coppiced and this variation also seems to depend on soil texture. The sandiest sites (i.e. LAV, MTL and STR) reached MYP faster than the clay site at ABI (Figures 1.3 and 1.4). For example, MYP at MTL1 was reached two years after coppicing or three years after SX67 establishment (i.e. S2R3). Conversely, soil compaction from extensive cultivation was exacerbated by the high clay content at ABI (Fontana, unpublished data). The time to reach MYP at that site was extended to three years after coppicing or four years after SX67 establishment (i.e. S3R4, Figure 1.3). The soils at the remaining sites were from loam to silty clay loam, excluding RXP which was dominantly organic. Their MYP were either reached after the third or the fourth growing season following SX67 establishment. Considering that SX67 in MTL1 and SJPJ1 is respectively the least and among the most productive and that MYP was reached at both SRCs three years after SX67 establishment (i.e. S2R3 and S3R3, respectively), the time to reach MYP appears independent of its magnitude.

As a whole, soil nutrient and water availability as well as soil physical properties are likely conditioning the dynamics of early willow growth and, in turn, the point in time at which a SRC reaches MYP. Based on our data, strong interactions with coppicing, stool establishment and soil texture are apparent. The time needed to reach MYP also appears to have an impact on yields over the various rotations. Kopp *et al.* (1993) argued that fertilization did not increase MYP, but rather decreased the time to reach MYP. On the contrary, N fertilization was reported to increase MYP, especially on acidic sandy soils (Labrecque & Teodorescu 2001). Kopp *et al.* (1993) conducted their study on relatively productive agricultural soils

so that plant nutrition was likely not a factor limiting growth. Fertilization had an impact on MYP, but it shortened the time needed to reach it, probably because the optimal availability of nutrients decreased the need for early root development (Heinsoo *et al.* 2009).

1.5.5 Effect of rotation on yields

Lower aboveground yields during the first rotation were frequently (Verwijst 1996) but not systematically observed (Lindegaard 2001; Nordh 2005). This pattern appears to be true on clayey soils at ABI and HTG, but does not seem to apply at BOI, STR or MTL where SX67 is supported by coarser soils (Figure 1.3, Tables 2 and 7). At SJPJ1 and the ABI site, the area of the largest diameter of the second rotation was larger than that of the first rotation (Figure 1.3, Table 1.7), probably due to the delay in reaching MYP. Within site, Larsen et al. (Larsen *et al.* 2014) found that aboveground biomass yields of the second rotation were quite homogeneous, independently of the large differences in yields observed for the first rotation due to the use of various methods for establishing and harvesting the SRC. This suggests that the increase in yields between the first two rotations is dependent of the yield of the first rotation.

Again at SJPJ1 and the ABI site, we speculate that a more established stool, which can access the soil resources more effectively, explains the lower estimated annual yields of the first rotation compared to those of the second rotation (Figure 1.3). In southern Sweden, an analysis carried out on more than 2,000 SRCs of willows indicated a significant increase in yields from the first to the second rotation (Mola-Yudego & Aronsson 2008). However, this study did not report a significant increase in yields from the second to the third rotation. Likewise, Labrecque and Teodorescu (2003) found that omitting to coppice *S. viminalis* and *S. discolor* led to a strong gain in yields between the first and second rotations. The differences in yields from the first to the second rotation could likely have been reduced if

coppicing had been conducted because it would possibly have allowed to reach the MYP one year earlier.

The estimated annual aboveground yield at LAV1 did not decrease after seven growing seasons (i.e. S7R9), nor did it decrease at HTG1 after four rotations with stools of fourteen years (i.e. S4R14, Figures 1.3 and 1.4). In fact, the largest diameters in the fourth rotation at HTG1 were higher than those of the previous rotations (Table 1.7). This is in full agreement with Volk *et al.* (2011) who reported some increases in yields between the first and second rotations (23%) and also between the first and fourth rotations (30.8%) for trials established on well-drained gravelly silt loam soils. However, the third rotation at HTG1 was among the least productive (Table 1.7). For nine willow cultivars, including SX67, Guidi Nissim *et al.* (2013) observed a higher annualized aboveground biomass production when accounting for a second rotation of four years compared to a first rotation or a third rotation of three years. Divergence was particularly large between the first and second rotations. Differences in annual biomass production could also be due to the fact that the first year is generally less productive than the second or third growing seasons, especially during the first rotation (Quaye & Volk 2011), and that adding a fourth productive growing season tends to increase the overall biomass production on an annualized basis. This observation is consistent with our findings because the SRCs with a fourth growing season were among the most productive (S4R5 for ABI1, S4R5 for ALB1, S4R8 for BOI1, S4R14 for HTG1, S4R6 for LAV1, S4R5 for MTL1 and S4R5 for RXP1, Figures 1.3 and 1.4).

1.6 Conclusion

Growth dynamics of SX67 was investigated as a function of stool age, coppicing and soil properties. Over the growing seasons, an asymptotic increase in annual aboveground yield is generally assumed until reaching maximum yields. The delay required to reach optimal annual yield affects the difference in aboveground

biomass yields between the first two rotations. Growth dynamics also strongly varied depending on site (soil) characteristics. Heavy clay soils restricted stool establishment. On compacted clayey soils, a lag of three years after coppicing was needed to reach maximum yields. At the sandiest sites, the delay to attain maximum yield was shortened to only two growing seasons after SX67 was coppiced, suggesting less constraining conditions for stool establishment. At sites where soils have a higher silt content (sandy loam or loam), the growth dynamics is expected to be somewhere between these two cases and the maximum yields can be high. In all soil types, aboveground biomass yields of the first growing season remained lower than subsequent growing seasons, especially during the first rotation. The difference was exacerbated at sites where soils restrict stool establishment (e.g. clayey soil with a plough pan). Our results do not support the idea that coppicing of SX67 should be performed for the sites that we tested with coarser soils because the positive effects on stem growth were negligible or inexistent, whereas productivity of stems growing on clayey soils was largely improved after coppicing. However, our data did not allow to test if total aboveground biomass yield was higher when combining the coppice year and the following three years of growth compared to a full four year rotation without coppicing. In the case that total aboveground biomass yield would be stronger by including coppicing, it would be valuable to perform the balance between the carbon gained by coppicing and the carbon cost to perform it.

CHAPITRE II

QUANTIFYING THE EFFECTS OF SOIL AND CLIMATE ON ABOVEGROUND BIOMASS PRODUCTION OF *SALIX MIYABEANA* SX67 IN QUEBEC

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2.1 Abstract

Short rotation cultures (SRC) of *Salix* are used for bioenergy purpose, but pedo-climatic conditions for optimizing aboveground biomass productivity are not precisely known. Using ten SRCs of *Salix miyabeana* SX67 growing in Québec, Canada, under heterogeneous conditions, we first identified and quantified the limitations induced by soil (across locations) and climate (across and within locations) on annual aboveground yields. We then compared the impacts that soil and climate have had on yields and developed pedo-climatic models of yields. Hence, soils and leaves were sampled in five plots per SRC. Soil bulk density, exchangeable cations and bulk composition as well as leaf $\delta^{13}C$ were analyzed. Climate during spring, summer and the whole growing season was simulated for each location using the BioSIM model. Water stress was estimated using leaf $\delta^{13}C$. Annual aboveground biomass yields of the SRCs were modeled using linear regressions, partitioning of the variance, mixed models and a multivariate regression tree with soil and climate as explanatory variables. Across SRCs, silt content, soil organic matter and soil exchangeable Ca and Mg levels were all significantly and linearly related to aboveground yields. Yield thresholds of organic matter, total P and spring temperature could also be computed. Annual yields were generally negatively related to drought within and across sites. This study reveals that soil variables had a greater impact on productivity than climate variables. Also, soil properties buffered or exacerbated climatic stress and thus, had a preponderant effect on yield.

Keywords: soil nutrients, pedo-climatic models, water use efficiency, site effect, farmland soils.

2.2 Introduction

Among thirty six applications with willows (Kuzovkina & Volk 2009), short rotation culture (SRC) was recognized as an alternative to reduce fossil fuels (Perttu 1999; Pacaldo *et al.* 2013). In southern Quebec, Canada, SRCs of willows (or *Salix*) are established given that they achieve high yields and have the potential to upgrade abandoned farmland (Labrecque & Teodorescu 2003). However, defining the optimal growing conditions for specific cultivars is still a major challenge for northern countries (Tahvanainen & Rytkönen 1999). The sites with the highest potential for *Salix* growth in such countries still remain poorly identified in part because it can grow relatively well under a large array of pedo-climatic conditions (Jørgensen & Blix 1985; Walker 1987; Pezeshki *et al.* 1998; Toillon *et al.* 2013). A wide range of productivity rates have also been reported for a given clone. For example, after one harvesting cycle of three years, the productivity of *Salix viminalis* varied between 10 t ha^{-1} in southern Finland (Tahvanainen and Rytkönen 1999) and 70 t ha^{-1} in southern Québec (Tahvanainen & Rytkönen 1999; Labrecque & Teodorescu 2003). The specific site conditions leading to these large variations in growth rates are not yet fully elucidated.

Salix species have very high nitrogen (N) requirements compared to other fast growing species such as *Populus* species (Jug *et al.* 1999). Also, growth of *Salix* cultivars are more sensitive to nutrient changes under SRCs than in natural environments (Weih 2001). Except after excessive addition of N (i.e. over 100 Kg ha^{-1}), yields of *Salix* are generally related to soil N contents (Ericsson 1981b; Labrecque *et al.* 1998; Toillon *et al.* 2013). Phosphorus (P) and potassium (K) were also shown to increase aboveground biomass production of *Salix* under SRCs (Ericsson 1981b; Adegbidi *et al.* 2001; Marler *et al.* 2001). However, these nutrients have not always been systematically positively correlated with growth after soil amendments (Labrecque & Teodorescu 2003). Under acidic soil conditions, *Salix* growth rates were increased following liming (Hytönen 2005). Similarly, at nine SRCs established across Canada on neutral to slightly alkaline soils, soil pH was positively linked to the productivity of *Salix purpurea* (Ens *et*

al. 2013). Hytönen (2005) suggested that root development of *Salix viminalis* was inhibited in soils with pH values below 4. Optimal pH range for *Salix* growth apparently ranges between 5 and 8 (Abrahamson *et al.* 2002), 6 and 7 (Mitchell *et al.* 1999) or 5.5 and 7 (Guidi *et al.* 2013). One possibility for the discrepancies in *Salix* growth relative to soil pH is that growth interacts with other variables such as soil texture and nutrients as well as harvesting cycles. On the one hand, *Salix* growth is generally higher in clayey soils compared to sandy soils (Labrecque & Teodorescu 2001; Toillon *et al.* 2013). On the other hand, soils can impede *Salix* productivity if it is too heavy (Guidi *et al.* 2013).

The impact of climate on *Salix* growth depends largely on the regional context. Dry conditions frequently limit the growth in low rainfall areas. This has been reported by Bergante *et al.* (2010) in Italy as well as by Ens *et al.* (2013) in Alberta and in Saskatchewan, Canada. In Greenland, snow precipitation was negatively related to *Salix arctica* growth (Schmidt *et al.* 2010). Water stress and water use efficiency (WUE) is linked to $\delta^{13}C$ values of plant tissues (Farquhar *et al.* 1982). When plant stomata are more closed to reduce transpiration in periods of low moisture, $\delta^{13}C$ values tend to increase, and vice-versa. A less negative value therefore means a higher WUE. On the one hand, $\delta^{13}C$ of *Salix miyabeana* wood suggested that the highest WUE was linked to greater biomass yields across three sites in Saskatchewan, Canada (Moukoumi *et al.* 2012). Ens *et al.* (2013) highlighted a similar relationship for *Salix purpurea* across a large climatic gradient from western to eastern Canada, although two sites with very high water stress did not follow the same trend as other less water stressed sites. Finally, Schifman *et al.* (2012) showed small $\delta^{13}C$ variations in *Salix* cultivars in tree rings, although the cultivars showing the largest variations were more resistant (higher survival) to drier conditions. It was therefore proposed that these cultivars could increase WUE when needed. Using *Salix* cultivars with such ability would likely be an advantage for SRCs, especially under climate change and potentially increasing drought events.

Growth of *Salix* in northern parts of North America or Europe is also largely limited by temperature. In Alaska, annual growth of *Salix lanata* was positively linked to temperature in mid-July and thawing degree-days (Walker 1987). In southern Finland, Tahvanainen and Rytkönen (1999) observed that biomass productivity of ten *Salix* cultivars was negatively related to May and September air temperatures and positively related with those of October. In northern Sweden, Wijk (1986) showed that shoot increments of *Salix herbacea* across years varied depending on growing season length. In turn, this influenced the amount of carbohydrates available for the following growing season. Also, in southern Sweden, biomass yield of *Salix viminalis* was modeled using total radiation (Sannervik *et al.* 2006). In Quebec, although a large number of SRCs exist, no study linking *Salix* yields to soil and climatic conditions was conducted.

The main objective of this paper was to identify and quantify the pedo-climatic factors limiting the growth of *Salix miyabeana* SX67 within and across ten SRCs in southern Quebec, Canada. A second objective was to develop a simple mathematical model which could be used by producers to determine the potential productivity of a site for SRCs of SX67.

2.3 Material and method

2.3.1 Field sites

This study was conducted at ten SRCs of *Salix miyabeana* SX67 in southern Quebec, which are characterized by different soil properties and climatic conditions (Tables 2.1-2.2, Figure 2.1). Geographical locations, site histories and field designs of each SRC are detailed in Chapter 1.

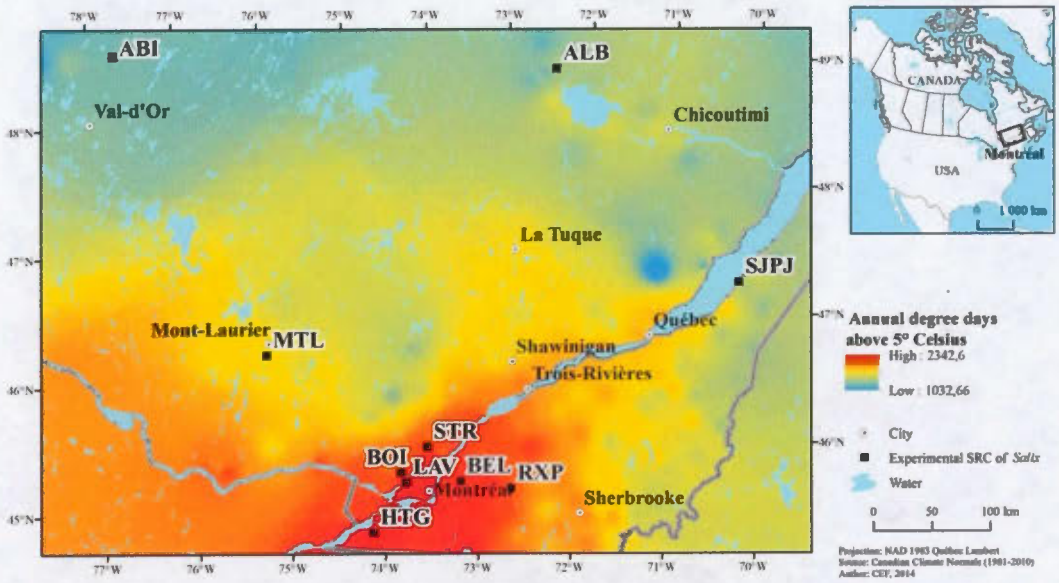

Figure 2.1 Location of the ten short rotation intensive willow cultures in southern Quebec and mean annual degree-days above 5°C in the study area.

Table 2.1 Soil particle size distribution and chemical properties at a depth of 0-25 cm and dry density at depths of 0-5, 20-25 and 40-45cm at each of the ten SRCs studied.

Site	Particle size			†O.M	pH	Total N and P		Exchangeable cations					Dry density		
	Sand	Silt	Clay			N_{tot}	P_2O_5	Ca_{exch}	Mg_{exch}	K_{exch}	Mn_{exch}	Al_{exch}	0-5	20-25	40-45
	——%——			–%–		—g kg^{-1}—		——cmolc kg^{-1}——					——g cm^{-3}——		
ABI	19.4	37.5	43.1	7.9	5.64	2.4	1.3	9.64	3.99	0.98	0.05	0.66	1.13	1.62	1.65
ALB	32.2	58.2	9.6	10.2	6.21	2.0	2.3	13.97	3.31	1.46	0.00	0.00	1.42	1.50	1.49
BEL	34.5	52.7	12.8	9.1	6.88	2.5	1.9	24.21	6.72	0.70	0.01	0.00	1.50	1.56	1.48
BOI	34.9	46.2	18.9	9.2	7.51	3.4	2.4	17.45	5.02	1.20	0.01	0.06	1.31	1.45	1.56
HTG	18.9	53.5	27.6	11.2	7.30	3.4	2.2	28.54	8.85	0.60	0.01	0.02	1.25	1.53	1.64
LAV	49.6	39.1	11.3	5.4	5.27	2.0	3.9	3.14	0.20	1.71	0.02	1.15	1.20	1.45	1.41
MTL	74.1	22.1	3.7	6.9	5.15	1.7	1.7	1.33	0.08	0.19	0.04	1.73	1.15	1.39	1.42
RXP	46.2	48.1	5.7	67.6	5.54	19.0	17.8	63.40	2.19	0.53	0.08	1.72	0.55	0.56	0.41
SJPJ	30.7	46.4	22.9	9.1	5.30	3.1	1.9	9.74	0.63	0.52	0.06	1.26	1.43	1.62	1.72
STR	59.2	32.1	8.7	4.6	5.60	1.7	1.6	5.14	0.78	0.69	0.02	0.34	1.47	1.68	1.71

† O.M. is organic matter

Table 2.2 Climate variables for each of the ten SRCs studied. Means are computed from the 2010, 2011, 2012 and 2013 growing seasons. The coefficients of variation are indicated in parentheses.

Site	Precipitation (mm)		†Drought (days)		Maximum temperature(°C)	Degree-Days (°C base 5)	‡MYP	MYP
	£Spring	£Summer	£Spring	£Seasonal	£Summer	£Seasonal		Mg ha^{-1} year^{-1}
ABI	202 (28%) a	192 (9%) a	10.8 (28%) a	11.5 (23%) a	30.4 (3%) a	1621 (3%) a	2011 to 2013	9.0 (11%)
ALB	225 (24%) ab	178 (50%) a	11 (43%) a	12.8 (26%) a	30.9 (6%) ab	1672 (3%) a	2012 to 2013	20.1 (1%)
BEL	334 (17%) c	185 (19%) a	10 (26%) a	11.3 (29%) a	32.9 (3%) b	2389 (3%) de	2013	28.0
BOI	289 (23%) abc	177 (12%) a	9.3 (23%) a	10 (14%) a	32.6 (4%) b	2358 (3%) de	2010 to 2013	18.6 (22%)
HTG	314 (20%) bc	177 (21%) a	9.8 (23%) a	10.5 (12%) a	32.3 (3%) b	2410 (3%) e	2011 to 2013	24.7 (39%)
LAV	278 (22%) abc	178 (21%) a	10.5 (29%) a	12.5 (33%) a	32.5 (3%) b	2477 (3%) e	2008 to 2013	13.1 (28%)
MTL	233 (31%) abc	186 (11%) a	12.3 (51%) a	13 (41%) a	32.0 (4%) ab	2049 (5%) c	2010 to 2013	6.5 (44%)
RXP	329 (18%) c	213 (32%) a	9.8 (26%) a	11.5 (21%) a	32.3 (3%) b	2336 (3%) de	2010 to 2013	15.2 (24%)
SJPJ	288 (30%) abc	162 (43%) a	9.3 (37%) a	12.8 (27%) a	31.5 (6%) ab	1885 (4%) b	2011 and 2013	21.3 (13%)
STR	309 (15%) bc	199 (15%) a	10.3 (37%) a	11 (26%) a	33.0 (3%) b	2293 (3%) d	2010, 2011	9.6 (4%)

† Refers to the highest value of consecutive days without precipitation.
‡ Indicates the growing seasons for which annual yield was considered to calculate the maximum yield potential (MYP) of the site.
£ Spring, summer and seasonal refer to the averages computed respectively from April to June, July to August and April to November.
Significant differences ($p < 0.05$, t-test) between SRCs and growing seasons are indicated within a column by different letters.

2.3.2 Estimates of annual aboveground biomass

At each site, five random plots (5 × 5 m) were delineated. Using the basal area increment, annual aboveground biomass yields were estimated for each plot and site using the approach described in Chapter 1. Once cuttings are planted, a few growing seasons are needed for the stools to fully establish and reach their maximum yield potential (MYP). We therefore modeled productivity only for growing seasons that had reached MYP. We also excluded from our models the first growing seasons of subsequent rotations because they were systematically lower than MYP (Chapter 1, Figure 1.3-1.4). These annual yields were averaged within each site to produce a set of MYP values that could be used to model productivity across sites. Because SRCs were not all established during the same year and the delay to reach MYP appeared to be influenced by soil properties (Chapter 1), the number of years used to compute MYP varied between SRCs.

2.3.3 Soil sampling and analysis

A soil sample was collected at both 0-25 cm and 25-50 cm in all 50 plots. Samples were air-dried and then sieved to pass through a 2 mm mesh. Soil particle size distribution was analyzed using the Horiba Partica LA-950v2 Laser Particle Analyzer (Horiba Instruments, Irvine, CA, USA). Samples from BOI, MTL and SJPJ were pre-treated to destroy sesquioxides. In this respect, samples were bleached twice with NaOCl (pH = 8, 1 hour at 25°C), centrifuged (400 rpm, 15 min) and finally washed with distilled water. Samples from RXP contained more than 60% of organic matter. Thus, the bulk of organic matter was first destroyed by loss on ignition and then treated with NaOCl before particle size measurement. Soil pH in water was measured using a soil:solution ratio of 1:2 for mineral samples and 1:10 for organic samples. Organic matter and inorganic C ($CaCO_3$) were measured on all samples by loss on ignition in a muffle furnace at 575°C (15 minutes) and 1000°C (10 minutes), respectively (Rabenhorst 1988). Exchangeable Ca, Mg, Mn, K, Na, Fe, Al and Zn (later referred as X_{exch}) were

determined by atomic absorption/emission (model AA-555, Agilent technologies, Santa Clara, CA, USA) after they were extracted using an unbuffered 0.1 M $BaCl_2$ solution (Hendershort *et al.* 2007b). Total soil organic C and N (N_{tot}) were determined only on 0-25 cm samples using the EA1108 CNHS-O Analyzer. Except for ALB and BEL, bulk composition (SiO_2, Al_2O_3, CaO, K_2O, MgO, MnO, Na_2O, P_2O_5, Fe_2O_3, Zn, TiO_2 and Ba) of all samples collected at 0-25 cm were analyzed by X-ray fluorescence spectrometry using a Philips PW2440 system (Panalytical, Almelo, The Netherlands) equipped with a Rhodium 60 kV end window X-ray source operating at 3 kW. The fused beads were prepared from a 1:4 soil:lithium tetra(meta)borate mixture which was heated for 18 min at 1000°C. For BEL and ALB, bulk chemical composition was also analyzed using X-ray fluorescence spectrometry (S8 Tiger WD XRF, Bruker, Billerica, MA, USA) but the fused beads were prepared from a 1:10 soil:lithium tetra(meta)borate mixture. Also, at three of the five plots within each site, an undisturbed soil core was collected (with a specially designed corer to perform shrinkage curves (Kohler-Milleret *et al.* 2013) at a distance of 20, 40 and 60 cm away from the shrubs and towards the inter rows. This procedure was performed at depths of 0-5, 20-25 and 40-45 cm for a total of 270 samples. Undisturbed soil samples were then air dried and the apparent density was measured following the plastic bag method (Boivin *et al.* 1990).

2.3.4 Climatic conditions

The BioSIM model was used to simulate climatic conditions at each SRC for all growing seasons covered in the study. Using site elevation, latitude and longitude, BioSIM uses multivariate regressions to extrapolate data from the closest climatic stations (Régnière 1996). Climatic variables (i.e. lowest temperatures, means of minimal temperatures (°C), means of temperatures (°C), means of highest temperatures (°C), highest temperatures (°C), degree-days (base 5°C), total solar radiation (MJ m^{-2}), precipitation (mm), means of number of days with precipitation, means of consecutive days with precipitation, highest values of

consecutive days with precipitation, means of number of days without precipitation, means of consecutive days without precipitation, highest values of consecutive days without precipitation) were modeled using the five closest stations to each SRC: (1) from the beginning of April to the end of June, referred as spring, (2) for July and August, referred as summer, and, (3) from the beginning of April to the end of November, referred as seasonal. BioSIM yields climatic data that are statistically indistinguishable from measured data (Régnière & Bolstad 1994).

2.3.5 Leaf sampling and analysis

At the end of August of 2011, foliage in the upper tier of the shrub was sampled on two contiguous healthy stools in each of the five plots at seven of the ten SRCs, i.e. ABI, BOI, LAV, MTL, RXP, SJPJ and STR (70 samples). Samples were oven dried at 40°C for at least 16h. Thirty leaves per sample were weighted before they were finely ground. A mean mass per leaf was calculated for each sample. Total C was analyzed using an elemental analyzer (Vario Micro Cube, Elementar, Hanau, Germany) which is coupled with a Micromass isotope ratio mass spectrometer (Isoprime 100, Isoprime, Cheadle, UK) in continuous flow mode to measure ^{12}C and ^{13}C. These isotopic C values were then expressed as $\delta^{13}C$ ‰ with respect to the international standard V-PDB using the following equation:

$$\delta^{13}C = \frac{R_{sample}}{R_{standard}} - 1$$

with R_{sample} and $R_{standard}$ being the $^{13}C/^{12}C$ ratio of the sample and standard, respectively. To correct the raw data due to the inherent fractionation of the analytical device, a calibration was built using two internal reference materials that were normalized to NBS19-LSVEC for $\delta^{13}C$: urea (-42.16‰) and sucrose (-11.85‰). A third internal reference material, leucine (-28.75‰), was included

with each batch of samples run on the mass spectrometer to assess the accuracy of the calibration.

Foliage samples were also digested with concentrated HNO_3 to determine Ca, K, Mg, Mn, Zn and Fe concentrations using atomic absorption/emission as indicated above. Phosphorus was analyzed colourimetrically (molybdate based method) from these digests using a QuickChem 8500 series 2 FIA System (Lachat Instruments, Loveland, CO, USA).

2.3.6 Statistical analysis

Datasets were arranged to allow statistical analyses at the site scale as well as at different temporal resolutions. First, to study the effect of soil and climate on productivity across SRCs, average soil and climate variables were computed for each SRC (referred as the site-scale dataset, n = 10). Climatic variables of each growing season for which annual yield values were used to compute MYP (Table 2.2) were also averaged at the level of the SRC to fit the site-scale dataset. Secondly, to examine the effects of climate variation on growth across growing seasons and SRCs, a dataset was built with all the annual yield values used to calculate MYPs (referred as the inter-annual dataset, n = 30). This dataset is unbalanced and data are not independent because we had to pool several growing seasons that were unevenly distributed for each SRC. The same soil variables as for the site-scale dataset were investigated. These variables were assumed to be constant over the years. Note that the BEL site was not included in this dataset because data was available for only one growing season at the site.

To visualize the variations of soil nutrient availability among plots and sites, a principal component analyses (PCA) was performed (with Euclidean distances between points conserved) using the function rda in the *vegan* package. The PCA was performed by directly using the plot level data (n = 50) to show the variability within rather than across SRCs.

T-tests were performed to compare climate variables of the 2010, 2011, 2012 and 2013 growing seasons between each pair of SRCs (n = 10). The conditions of normality and homoscedasticity were graphically checked for all models (i.e. parametric linear models).

Water use efficiency (WUE) and water stress during the 2011 growing season were investigated using foliar $\delta^{13}C$ values. T-tests were performed between all possible pairs of SRCs to verify if $\delta^{13}C$ values significantly differed ($p<0.05$). Using the data for the 2011 growing season only, a forward selection was performed to detect if climate variables were related to foliar $\delta^{13}C$ values across SRCs. Foliar $\delta^{13}C$ at ABI and LAV was dramatically high and low when compared to the amounts of precipitation received in 2011. Foliar $\delta^{13}C$ at these two sites clearly responded differently to climatic variables compared to the other sites due to specific soil conditions (see Discussion). Therefore, a linear regression was built between foliar $\delta^{13}C$ and the climate variables previously selected with the forward selection procedure, but ABI and LAV were omitted from the analysis.

To test for the effect of climate on productivity within sites and across growing seasons, forward selections were first performed separately for each SRC. This allowed detecting climate variables that best explained annual yields. Except at the LAV site (n = 6), the number of data points was too low to draw robust regressions. However, we grouped the data of sites whose significant climate variables were collinear. This allowed building a mixed model with summer degree-days (5°C) and sites as fixed and random factors, respectively. Analyses were performed using the function lme available in the *nlme* package. This function is quite robust to model unbalanced designs (Laird & Ware 1982).

To identify (1) soil variables and (2) climate variables that best explained MYP values across SRCs, forward selections were performed across sites. This was carried out using the function forward.sel in the *packfor* package. Chemical properties of the organic soil at the RXP site were dramatically different

compared to those of the mineral soils at all other sites, and the ABI site showed evidence of water shortages compared to other sites (i.e. $\delta^{13}C$ was dramatically less negative compared to all other sites, see Figure 2.3 and Discussion). Therefore, various regression models were built, i.e. with and without the RXP data for regressions using soil variables, and with and without the ABI data for regressions using climate variables.

For a better insight on the effects of soil on productivity compared to those of climate, the variance of MYP was partitioned using both soil and climate variables that best explained MYP (i.e. silt and spring drought) (Peres-Neto *et al.* 2006). Partitioning was done using the function varpart available in the *vegan* package. Variances that were explained only by silt or spring drought were tested by permutation through partial canonical redundancy analysis. The function rda available in the *vegan* package was used. The effect of spring drought on productivity which was also explained by silt was considered as an overestimation that occurred by chance due to the distribution of SRCs in our network (i.e. low silt content and high spring drought that are unfavorable for growth were found within the same SRCs, see Discussion). Thus, the pedoclimatic model developed to estimate growth prioritized the soil effect. In this respect, the MYP:silt content ratio was computed first. The linear relationship between this ratio and drought was then tested – it yielded an equation capable of approximating MYP. Modeling was performed without the SRC showing significant water shortages (i.e. ABI in this study), as similarly carried out by Ens *et al.* (2013).

Finally, to compare the effect of site and climate on annual yields, a multivariate regression tree (MRT) was performed by considering all sites and growing seasons. This non parametric method of hierarchical clustering was used to model annual yields as a response variable and soil and climate variables as explanatory variables (De'ath 2002). The MRT allowed to sort by order of importance the successive thresholds of soil and climate variables able to explain the variance in annual yields. The data set was recursively split in two clusters whose sums of least squares of the response variable were minimized. The output of the MRT

also provided a residual error, which yields the R^2 of the model (i.e. 1-error). The MRT was performed using the function mvpart available in the *mvpart* packages. Some didactic explanations and examples are described in Legendre and Legendre (2012) and Borcard *et al.* (2011).

2.4 Results

2.4.1 Soil and climate conditions

The SRCs included in this study are characterized by heterogeneous soil conditions (Table 2.1, Figure 2.2). Soils from nine SRCs are developed from mineral parent materials, whereas the RXP soil is developed from organic material. Therefore, the RXP soil has very high exchangeable Ca (Ca_{exch}), organic matter content and a dramatically low apparent bulk density (Table 2.1) compared to other SRCs. Soil pH across the SRCs ranges between 5.1 and 7.5. The coarser (sandiest) soils are generally the most acidic and have the highest Al_{exch} (Table 2.1). Soil P_2O_5 at the LAV site is the highest among the SRCs supported by mineral soils. Variability in climate as related to precipitation and maximum summer temperature is mostly influenced by the growing seasons rather than the sites, whereas the opposite is observed for variables related to degree-days (5°C) (Figure 2.1, Tables 2.2-2.3). The ABI and ALB sites are the coldest, followed by the SJPJ and MTL sites. As a whole, climatic conditions are similar between the rest of the six more southerly sites.

Table 2.3 Climate variables for individual growing seasons. Means are computed from the ten SRCs studied. The coefficients of variation are indicated in parentheses.

Year	Precipitation		Drought		Maximum temperature	Degree-Days
	Seasonal (Days)	Summer (mm)	Spring (days)	Seasonal (days)	Summer (°C)	Seasonal (°C base 5)
2010	95 (10%)	176 (23%) a	14 (10%) a	14.0 (10%) a	33.0 (3%) a	2161 (14%) a
2011	93 (7%)	231 (19%) b	7.8 (21%) b	10.1 (14%) b	31.5 (6%) bc	2190 (15%) a
2012	78 (13%)	150 (16%) a	10.8 (34%)	13.8 (25%) a	32.7 (2%) ab	2193 (15%) a
2013	95 (10%)	181 (14%) ab	8.5 (14%) b	8.8 (7%) c	30.9 (2%) c	2051 (15%) a

Spring, summer and seasonal refer to the averages computed respectively from April to June, July to August and April to November.
Significant differences ($p < 0.05$, t-test) between SRCs and growing seasons are indicated within a column by different letters.

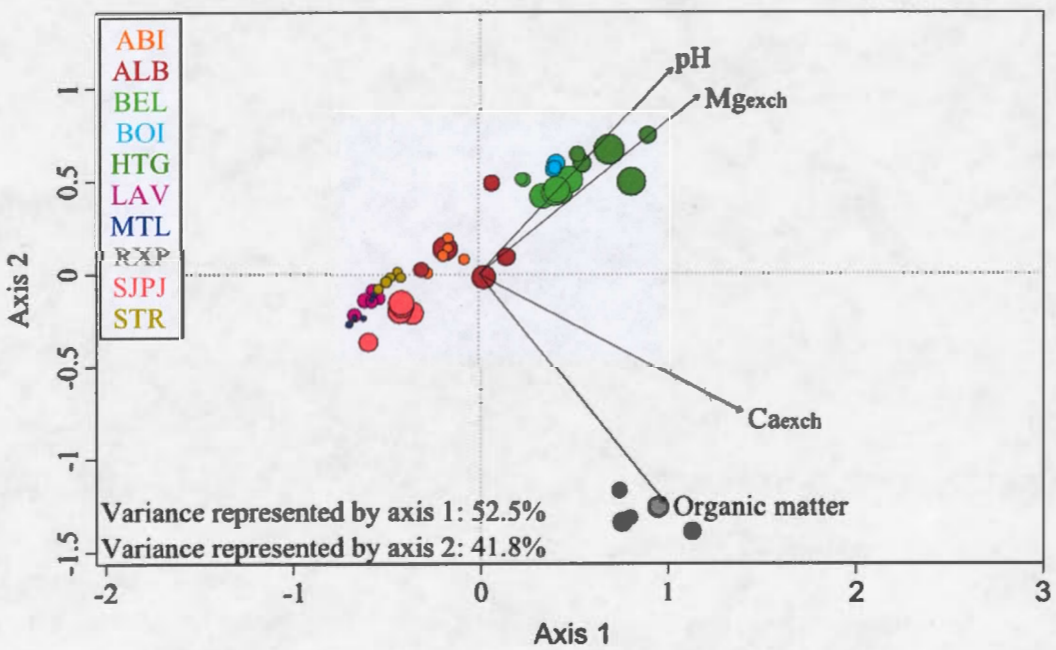

Figure 2.2 Principal component analysis of soil organic matter, exchangeable calcium and magnesium (Ca_{exch} and Mg_{exch}) and pH. Circle size is proportional to the maximum yield potential.

2.4.2 Site productivity as related to soil

Whether all SRCs or only the SRCs with soils developed from mineral parent materials (thus excluding RXP) are included in the analysis, the silt content is the best proxy of productivity (Table 2.4). Also, Mg_{exch} concentration is a significant indicator ($P \leq 0.1$) of productivity regardless of parent material type. In soils developed from mineral parent materials, organic matter, Ca_{exch} and total Zn concentrations are also related to productivity. However, the strength of the relationship decreases notably when the data from RXP site are included in the analysis. For mineral soils, at a depth of 25-50 cm, only Ca_{exch} concentration is significantly linked to MYP. Note that if soil nutrient concentrations are multiplied by the apparent soil bulk density to obtain a volumetric unit (per m^3) to reflect the availability of nutrients in a specific soil volume explored by roots, the relationship between MYP and total N content is significant (adjusted $R^2 = 0.45$, $p < 0.05$) for soils developed from mineral parent materials. Also, significant relationships exist between MYP and soil Ca_{exch} content with (adjusted $R^2 = 0.54$, $p < 0.01$) and without (adjusted $R^2 = 0.63$, $p < 0.01$) the RXP data.

Table 2.4 Significant linear regressions between soil properties and maximum yield potential across the ten SRCs studied. Adjusted R^2 are presented and positive (+) and negative (-) relationships are indicated in parentheses.

Soil depth	n = 10	Adj. R^2	n = 9	Adj. R^2
0-25 cm	(+) Silt**	0.72	(+) Silt**	0.72
	(+) $Mg_{exch}$$^{(*)}$	0.25	(+) O.M.*	0.41
			(+) Ca_{exch}**	0.54
			(+) $Mg_{exch}$$^{(*)}$	0.25
			(+) Zn_{tot}*	0.37
			(-) $Zn_{exch}$$^{(*)}$	0.27
			(-) $Fe_{exch}$$^{(*)}$	0.26
25-50 cm			(+) Ca_{exch}*	0.31
			(+) $Fe_{exch}$$^{(*)}$	0.26

For n = 9, models were performed without the RXP data.
Linear models are significant at $p \leq 0.1$ (*), $p \leq 0.05$ (*) and $p \leq 0.01$ (**).
exch and tot refer to exchangeable and total, respectively. O.M. is organic matter.

2.4.3 Foliar $\delta^{13}C$ during the 2011 growing season

At the ABI site, foliar $\delta^{13}C$ during the 2011 growing season was significantly less negative than all other SRCs (Figure 2.3a). The BOI and MTL sites also tended to have less negative values than the LAV, STR, SJPJ and RXP sites. For these sites, foliar $\delta^{13}C$ values suggest that the shrubs have the lowest WUE among all shrubs studied. Moreover, when removing the data from the ABI and LAV sites, foliar $\delta^{13}C$ is significantly explained by summer precipitation (Figure 2.3b). However, across SRCs, no relationship is found between annual yield of the 2011 growing season and foliar $\delta^{13}C$, whether the data from the ABI and LAV sites is included or not (results not shown).

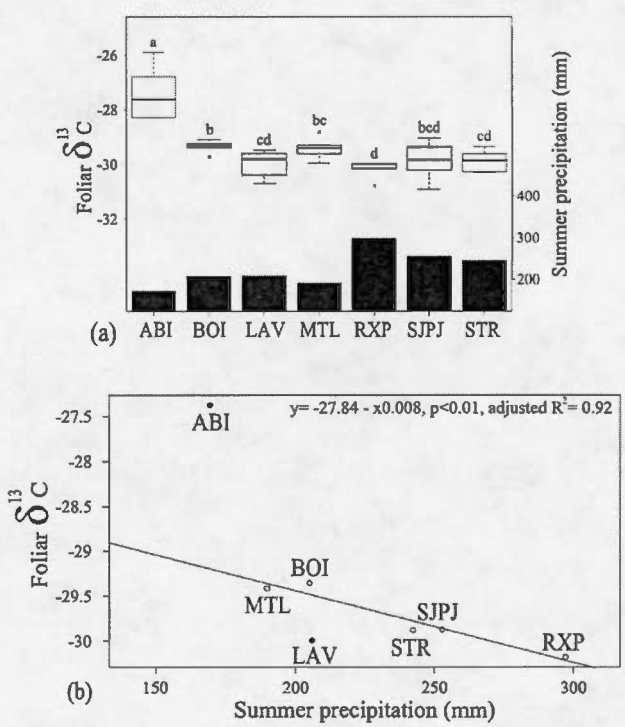

Figure 2.3 Mean foliar $\delta^{13}C$ (boxes) and summer precipitation (bars) measured for the 2011 growing season at each SRC studied (a) and linear regression between foliar $\delta^{13}C$ and summer precipitation without considering the data from ABI and LAV sites (b). In panel (a), different letters were assigned when a significant mean difference ($p<0.05$) was found between SRCs.

2.4.4 Site productivity as related to climate

At the LAV site, there is a positive relationship between the number of days with precipitation (i.e. from April to November) and annual yields ($R^2 = 0.71$, $p < 0.01$, Table 2.5). Conversely, significant relationships between annual yields and climate variables at other SRCs suggest a negative impact of air temperature on growth. Therefore, mixed models encompassing all SRCs except the LAV site are significant ($R^2 = 0.89$, $p < 0.05$, Table 2.5) and negatively linked to summer degree-days.

Table 2.5 Mixed model with annual aboveground biomass yield as the response variable, site as a random factor and the mean of the maximum temperature as a fixed variable. The adjusted R^2 of the linear regression between summer drought and annual aboveground yield at the LAV site is indicated in the shaded area of the table. Positive (+) and negative (-) relationships are indicated in parentheses.

	Mixed model		
Sites	**n**		**R^2**
ABI, ALB, BOI, HTG, MTL, RXP, SJPJ, STR	24	†Summer degree-days (5°C)*(-)	0.90
LAV	6	Annual days with precipitation**(-)	0.71

†Summer refers to the average of July to August.
Linear models are significant at $p \leq 0.05$ (*) and $p \leq 0.01$ (**).

Across SRCs, MYP is significantly related to climate variables that are linked to drought (Table 2.6). The strength of the relationships increases when the data from the ABI site are excluded.

Table 2.6 Significant linear regressions between climate variables and maximum yield potential across SRCs. Adjusted R^2 are presented and negative (-) relationships are indicated in parentheses.

Climatic variables	**All SRCs (n = 10)**	**Without ABI (n = 9)**
†Spring drought	0.63**(-)	0.74**(-)
£Seasonal drought	0.49*(-)	0.65**(-)

†Spring refers to the averages computed from April to June.
£Seasonal refers to the averages computed from April to November.
Drought refers to the highest value of consecutive days without precipitation.
Linear models are significant at $p \leq 0.05$ (*) and $p \leq 0.01$ (**).

2.4.5 Pedoclimatic models of productivity

The variance in MYP is successfully partitioned with two explanatory variables, i.e. silt content and spring drought. These are the most robust soil and climate variables detected. They are also easily measured in the laboratory (silt) or estimated with BioSIM (drought). A strong collinearity between the two variables is present (Figures 2.4 a,b).

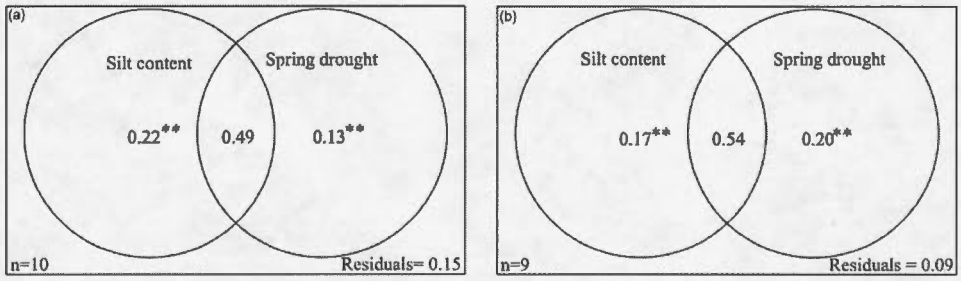

Figure 2.4 Partitioning of the variance of maximum yield potential (MYP) with silt content and spring drought (i.e. the highest number of consecutive days without precipitation between April and June) as explanatory variables with (a) and without (b) the data from the ABI site. The rectangles represent the variance of maximum yield potential (MYP), circles represent the variance explained by the two explanatory variables and the overlapping area between circles shows the variance concomitantly explained by both variables.

Also, the variance in MYP explained by drought increases when the data from the ABI site were removed. However, in all cases, each variable explained a significant part of the variance that was not explained by the other variable. After detrending for the silt effect, we find that a robust pedo-climatic model (linear regression without the data from the ABI site) can be developed to simulate MYP for nine of the ten sites (adj. $R^2 = 0.92$, $p < 0.001$, Figure 2.5).

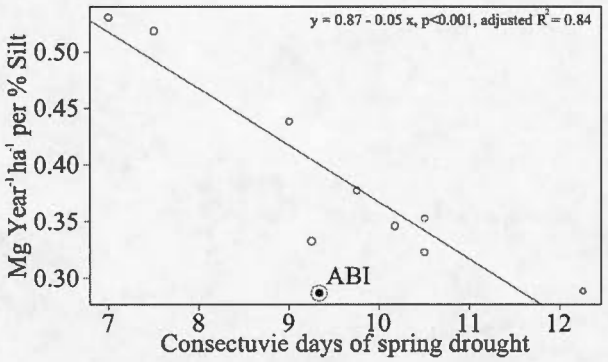

Figure 2.5 Linear regression between the MYP:silt ratio and consecutive days of spring drought is shown without considering the ABI data point (highlighted by a red circle). The relationship between MYP and silt and spring drought is fully detailed in the Material and Method section.

A second pedo-climatic model was developed using MRT (Figure 2.6). In this case, among all soil and climate variables, a threshold of soil organic matter best explained annual yields (R^2 = 0.59). This first threshold separates the most productive sites (i.e. ALB, HTG, RXP and SJPJ) from the least productive sites (i.e. ABI, BOI, LAV, MTL and STR). Annual yields of these two clusters are separated again by soil P_2O_5 and the spring minimal temperature, respectively.

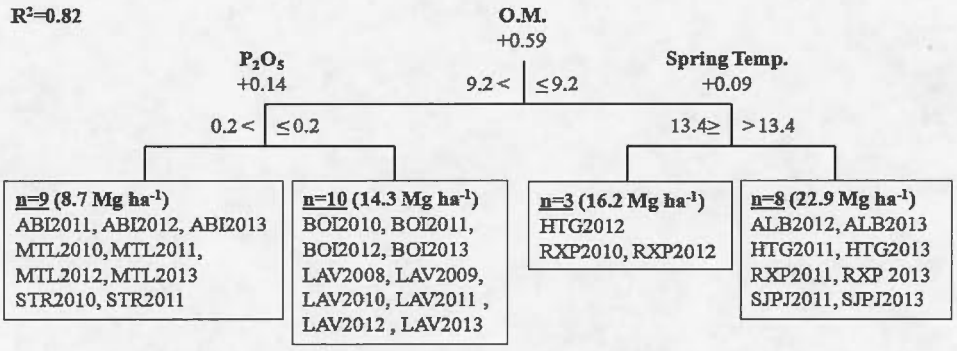

Figure 2.6 Variance of annual aboveground biomass yields explained by soil and climate variables through a multivariate regression tree. The additional contributions to the model R^2 (i.e. 1 – residual error) are presented for each node. The number following the site name (e.g. ABI2011) refers to the growing season for which annual yield is considered. For each cluster, the mean annual yield is reported in bold. O.M. is soil organic matter, P_2O_5 is soil total P, and Spring Temp. is spring temperature averages computed from April to June.

2.5 Discussion

2.5.1 Soil variables as related to yield

The reliability of soil Ca_{exch} concentration as a proxy to estimate growth was robust only when the data from the RXP site was omitted from the model (Table 2.4). This is due to RXP being developed on an organic soil which is characterized by the highest Ca_{exch} concentration despite exhibiting yields just above average the SRCs network studied (Table 2.1). The low soil bulk density at the RXP site resulted in a dramatic decrease of Ca_{exch} concentration when it was calculated volumetrically. This variable was significant for modeling the productivity of all sites, i.e. including RXP. The volumetric concentration was also a better predictor of productivity than mass concentration when only sites with mineral soils were considered. This is likely explained by the fact that soil Ca availability is better estimated when considering a soil volume, explored by roots, rather than a soil mass. The high acidity of some of the soils in our network, formerly forest soils with pH values nearing 5.0, results in high soil exchangeable Al_{exch} saturation and thus much lower Ca_{exch} concentrations (Table 2.1). As in Ens *et al.* (2013) for *Salix purpurea*, we found that soil chemical properties in the upper part of the profile best explained productivity across SRCs (Table 2.4). This is probably because fine roots of *Salix* are generally concentrated within the first 10 cm of soil (Heinsoo 2009; Jerbi *et al.* 2015). Similarly, Ens et al. (2013) found a very strong positive relationship between yields and soil Ca_{exch} concentration, although the relationship was stronger with total CaO content and pH, indicating a significant influence of the nature of the parent material (e.g. acid-base status) on growth.

Soil Mg_{exch} concentration also explained about 25% of the variance in *Salix* growth whether the data from the RXP site was included or omitted from the analysis (Table 2.4). Despite being an essential nutrient (Ericsson 1981b), there is no study, to our knowledge, reporting a positive relationship between soil Mg_{exch} concentration and yields of *Salix*. This positive relationship is likely explained by

the fact that less productive SRCs have both high sand content and low Mg_{exch} concentration (Table 2.1). The SRCs supported by sandy soils (i.e. LAV, MTL and STR) were also characterized by low soil pH. In the range of soil pH values determined in our study (5.15 to 7.51, Table 2.1), both nitrification rates and base cation availability can increase significantly with rising pH (Havlin *et al.* 2005), while solubility of trace metals such as Zn or Mn can decrease (Sanders 1983; Martínez & Motto 2000). Optimum pH ranges were often assessed for *Salix* spp., but these varied between studies (Mitchell *et al.* 1999; Abrahamson *et al.* 2002; Guidi *et al.* 2013). With our data, it is difficult to set minimum and maximum pH thresholds because the SJPJ and RXP sites were among the most productive sites and had relatively low pH values (5.3 and 5.5, respectively), suggesting that soil pH had no direct impact on SX 67 growth.

In this study, soil Mg_{exch} and Ca_{exch} concentrations and silt content were collinear across sites characterized by mineral soils (results not shown). Ens *et al.* (2013) reported that the most productive SRCs of *Salix purpurea* had a silt content ranging from 40 to 60%, which is equivalent to a loamy soil. Similarly, Guidi *et al.* (2013) suggested that loamy soils are ideal for optimal *Salix* productivity, whereas Tahvanainen and Rytkönen (1999) found that productivity of *Salix viminalis* was decreased on coarse textured (sandy) soils or heavy compacted clays. In our study, this proxy of soil particle size distribution was also quite robust to estimate growth even when the minor mineral fraction of the RXP soil was included in the analysis (Table 2.4). The structured nature of loamy soils apparently favours gas and water flow while ensuring water retention (Pachepsky *et al.* 2001).

2.5.2 Factors limiting yield at low productivity sites

At SRCs where soils are characterized by coarse textures, i.e. LAV, MTL and STR, soil nutrient availability is generally low, e.g. total N and Ca_{exch} (Table 2.1, Figure 2.2). This likely explains the low productivity at the MTL site. At the STR

site, we suspected that the Ap horizon had been depleted in C and some nutrients due to a history of intensive farming and tilling which decreased organic matter and soil stability (Lipiec & Stępniewski 1995). A preliminary analysis of three soil profiles of the adjoining grass trips, which were not cultivated or at least not as intensively in the past (as indicated by the absence of a plough pan), confirmed our hypothesis. Clay content, organic matter levels, exchangeable Ca and Mg concentrations and pH were dramatically higher in the A horizon of the grass strips (+118, 57, 45 and 106%, respectively) compared to that of the cultivated soil (results not shown).

Also, repeated tilling was reported to increase bulk density until a depth of 40 cm on clay loam and sandy loam soils, which strongly impeded the productivity of *Salix viminalis* during the first growing season (Souch *et al.* 2004). The following years, an increase in growth was observed likely because the root system had developed beyond the compacted layer (up to a depth of 2 m). At the ABI site, a plough pan at a depth of about 20 cm (Table 2.1, Figure 2.7) is evidence of soil compaction and this appears to have completely restricted root development beyond that depth. Consequently, this was the only SRC for which soil bulk density at 0-5 cm increased with the distance between the inter row and the collected sample (i.e. overall, bulk density increased by 7.3% from 20 cm to 60 cm away from the row, not shown). Hence, the roots were constrained to a shallower soil volume for nutrient prospection and acquisition. This also increased the sensitivity of the root system to drought as seen by less negative foliar $\delta^{13}C$ values, indicating a greater water shortage at ABI during the 2011 growing season despite being a relatively high rainfall year (Table 2.3). Low productivity at the ABI site was therefore likely induced by soil compaction that eventually led to a smaller soil volume for roots to explore and water stress. For this reason, it was decided to model SX67 yields without the ABI data for some variables (Figures 2.3-2.4, Table 2.6). Martin and Stephens (2006) proposed that increasing soil bulk density decreased the yield of *Salix viminalis* as waterlogging was induced by the low soil aeration. Establishing SRCs of *Salix* at sites where conventional tillage

was performed on a yearly basis, especially on clayey soils, is also to be avoided as this can decrease nutrient and water availability as well as microbial activity (Gadermaier *et al.* 2012; Fontana *et al.* 2015). Former agricultural land may create restrictions for optimal *Salix* growth and it is therefore suggested that plough pans be mechanically destroyed before SRCs establishment (Borghei *et al.* 2008). In turn, *Salix* root development could restore some structure to the soil as seen with *Alnus glutinosa* (Meyer *et al.* 2014).

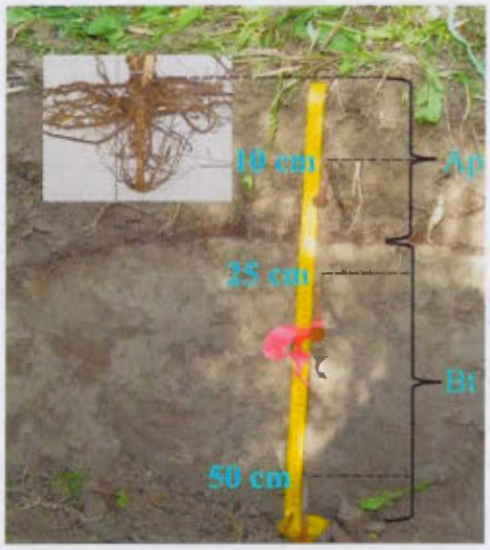

Figure 2.7 Typical soil profile at the ABI site where the dominant upward development of willow roots is due to the presence of a plough-pan at a depth of 20 cm. The embedded picture of willow roots was taken while assessing biomass of the whole rooting system in the laboratory for six of the SRCs studied (Tremblay 2014).

2.5.3 Water stress across growing seasons and SRCs

In high-latitude forest systems, air temperature is generally positively linked to productivity when soil moisture is sufficient, but the relationship can become negative under water deficits (D'Arrigo *et al.* 2008). At the LAV site, the negative relationship between yield and precipitation, which followed roughly the same annual fluctuation as seasonal degree-days (see Table 2.3), suggests that this SRC did not undergo water limitations (Table 2.5). This is likely because the sandy

loam soil at site allows for the development of a deep rooting system reaching at least 70 cm (M. Fontana, personal observation). Also, a perched water table and/or poor water infiltration at many points in time during the growing season was visible from water pools at the soil surface, thus reflecting a status of recurring high soil moisture (M. Fontana, personal observation). Similarly in Svalbard, Norway, there was no indication of water shortage as the growth of *Salix polaris* was negatively correlated with summer precipitation (Buchwal *et al.* 2013). The authors argued that the negative effect of rainfall on growth was probably due to a concomitant decrease of air temperature and solar radiation. Summer precipitation and air temperature at the LAV site were similar to that of the BOI site; however, foliar $\delta^{13}C$ was significantly more negative at the LAV site compared to the BOI site (Figure 2.3). These results highlight the strong influence of soil type on WUE and water stress (Martin & Stephens 2006; Moukoumi *et al.* 2012).

Except at the LAV site, yields within all other sites were negatively impacted by summer degree-days (Table 2.5). Increased degree-days likely triggered more frequent episodes of water stress. Such episodes have been linked to declines in tree growth in Quebec (Payette *et al.* 1996; Wheaton *et al.* 2008). Similarly, Guidi Nissim *et al.* (2013) suggested that the coupling of a relatively low seasonal rainfall and high air temperature were responsible for the low annual yields of various *Salix* cultivars in southern Quebec. Conversely, the negative response of tree growth to precipitation at the LAV site is the reason why it had to be modeled separately (Table 2.5). Sevel *et al.* (2012) observed higher yields of four *Salix* cultivars on sand compared to organic soil for a particular year in Denmark, whereas the opposite was observed the following year. The yields at these sites could have varied due to changes in soil water status such as episodes of water logging at the LAV site.

Across SRCs in Canada without high water deficits, a positive relationship was observed between foliar and wood $\delta^{13}C$ and yields of *Salix purpurea*, suggesting that WUE (and thus water availability) did not limit yields (Moukoumi *et al.*

2012; Ens *et al.* 2013). In our study, no such relationship was found for the 2011 growing season (results not shown), whether the data from the ABI and LAV sites were omitted or not from the analysis. However, our results suggested that summer precipitation mainly affected WUE across the SRCs for the 2011 growing season when the ABI and LAV data were removed from the regression analysis because WUE at these sites behaved differently due to intrinsic soil conditions (Figure 2.3b). When all sites were computed, a logarithmic regression was produced (adjusted $R^2 = 0.50$, $p < 0.05$). However, the relationship was not considered to be biologically meaningful given that the dramatically low foliar $\delta^{13}C$ at ABI was mainly due to soils exacerbating water stress for SX67. Except at the ABI site and perhaps for the 2012 growing season which was a very hot and dry summer in Quebec (Table 2.3), water stress was apparently not large enough to significantly limit productivity across sites during the 2011growing season. Nevertheless, when climate variables were averaged on an annual basis, spring drought partly limited productivity (Table 2.6, Figure 2.4). It should be noted that even if spring drought was the most robust climatic variable to explain yields, seasonal drought also explained 49% of the variation in yield ($p < 0.05$, Table 2.6).

Despite the strong gradient in degree-days (Table 2.2), no relationship with yield was detected. One of the two northernmost SRCs is among the least productive (i.e. ABI), whereas the second is among the most productive (i.e. ALB). Clearly, and as discussed above, there are more reliable indicators of growth than degree-days. In contrast, along a latitudinal transect in the clay belt of Quebec, Lapointe-Garant *et al.* (2010) found that seasonal degree-days was the only climatic variable with large enough variations across sites to influence the growth of *Populus tremuloides* stands. We suspect that soils were more homogeneous, although this information was not provided.

2.5.4 Pedoclimatic yield models

The productivity within sites and across the years was only influenced by climatic conditions and varied by up to 44% (Table 2.2). In contrast, the productivity across SRCs varied by a maximum of 380% (data not shown) and was influenced by soil and climate. Since climatic variables limiting yield significantly differed between years and not between sites (Tables 2.2-2.3), we therefore assumed that soil was a more important limiting factor of *Salix* growth compared to climate. When quantitatively confronting the effects of climate and soil, we found a strong collinearity between silt content and frequency of drought episodes (Figure 2.4). This is because soils with unfavorable growing conditions were among the driest (i.e. ABI, MTL), whereas those with optimal growing conditions (i.e. BEL, HTG, SJPJ) had the greatest moisture levels (Table 2.2). When including or omitting the data from the ABI and LAV sites, seasonal drought explained respectively 13 and 20% of the variance in yields that was not explained by silt content. It therefore appears more realistic and unbiased to consider only this fraction for the effect on climate on yields instead of the whole climate effect (i.e. including the collinearity) which is in the same order of magnitude as that of the soil effect (Figures 2.4 a,b).

In this respect, a model was developed to forecast the potential of productivity without overestimating the drought effect (Figure 2.5). Except under severe drought conditions such as Mediterranean climates (Bergante *et al.* 2010), the soil has generally a stronger impact on the productivity of *Salix* under SRC than climate (Weih 2004; Ens *et al.* 2013). The MRT suggested a similarly larger role of soil ($R^2 = 0.73$, Figure 2.6) on *Salix* productivity than climate ($R^2 = 0.09$, Figure 2.6). It explained 82% of the variance in annual yields, whereas forward selection failed to find a significant relationship. This shows the utility of MRT for such a context and confirms the relevance of soil organic matter as a proxy to estimate SX67 yields. Even if the thresholds detected by the MRT make sense, pedo-climatic models of productivity are limited by the range of more extreme data (Basso *et al.* 2013). The MRT suggests a threshold of mean air temperature for

spring, which splits the annual yield values of the most productive sites (i.e. with the higher soil organic matter content), thereby highlighting that the lowest yields were observed during the warmer growing seasons (i.e. 2010 and 2012, Table 2.3, Figure 2.6). However, the results obtained by the MRT and linear models using the various datasets to explain aboveground biomass yields are consistent in regard to the effect of soil and climate.

2.6 Conclusion

Within each of the SRCs studied, different factors limited aboveground biomass yields of *Salix miyabeana* SX67. Soil nutritional balances and soil physical properties, which were likely influenced by past agricultural practices, appeared as significant factors. To estimate the maximum potential biomass yield of a site, silt content was a reliable indicator as it offers high cation exchange capacity without placing the same mechanical constraints for root growth compared to clay soils, especially those compacted by tilling. Also, linear and non parametric approaches highlighted that soil properties are the most robust indicators of productivity (i.e. 60-70%), whereas climatic conditions explain yields to a lesser extent (i.e. 10-20%). This is partly due to the larger heterogeneity of soils compared to that of climate in southern Quebec. However, this study confirms that water stress limits the productivity in this area. Under a warming climate, evapotranspiration rates and thus water deficits should increase in southern Quebec. Due to its capacity to accumulate soil organic carbon relative to previous land use (Kahle *et al.* 2007; Lafleur *et al.* 2015), *Salix* grown under SRC should increase nutrient pool and water holding capacity after several growing seasons, but this depends on initial soil properties. An increase in soil organic carbon alleviate, in part, water shortages under the changing climate. Proper care should be taken at all stages of SRC management to optimize soil organic matter and hydro-structural properties.

CHAPITRE III

LEAF CHEMISTRY AND MASS EXPLAIN INTER-ANNUAL VARIATIONS IN *SALIX MIYABEANA* SX67 YIELD IN QUEBEC

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3.1 Abstract

- *Aims:* Compare the effects of soil properties and of climate variations across three growing seasons on foliar nutrition and mass of nine *Salix miyabeana* SX67 short rotation cultures established over a large geographic range in Quebec, and develop models to predict annual aboveground biomass yields using leaf chemistry and mass.
- *Methods:* Leaf samples were collected over three growing seasons at the nine sites. For each site, annual productivity and climate were estimated, and general soil properties (i.e. particle size distribution, organic matter, exchangeable cations, bulk chemical composition, including N and P_2O_5), leaf mass and foliar nutrients/elements (i.e. C, N, P, K, Ca, Mg, Mn, Zn, and Fe) were assessed.
- *Results:* Although climatic variations across growing seasons were large, specific foliar signatures were largely imposed by the soil chemical footprint of the site. Foliar N, K, Ca, Mg, Mn and Zn were related with their respective soil concentrations (total or exchangeable), whereas foliar C, N, K, Ca and Mg were linearly related to climate. Annual biomass yields were linearly related to leaf N, Ca, Mn and mass. However, the magnitude of impact of these variables on growth depended on the environmental conditions during the growing season. Annual biomass yields were better explained by non-linear than linear relationships.
- *Conclusions:* This study emphasizes that the foliar signature of *Salix miyabeana* SX67 is more dependent of differences between sites than growing seasons, but nutrients limiting growth can also change depending on growing season. Forecasting yields using thresholds of foliar nutrients allowed accounting for this last effect. It was therefore possible to build models that produce highly reliable yield estimates.

Keywords: leaf traits, willow, foliar nutrients, aboveground biomass, partitioning of variance, multivariate regression tree.

3.2 Introduction

Despite the recent decline in oil prices, biomass energy sources such as those produced from willow (*Salix*) short rotation cultures (SRC) still represent a strategic interest for several countries in Northern Europe as well as in some states and provinces of the US and Canada to reduce their dependency on fossil fuels. *Salix* spp. have a high physiological and growth plasticity (He & Dong 2003) and can be cultivated from an array of soil and climatic conditions (Tahvanainen & Rytkönen 1999; Sannervik *et al.* 2006; Aylott *et al.* 2008a; Ens *et al.* 2013). However, productivity of *Salix* within the same species or genotype can vary substantially from one site to another. Genotypes of *Salix* grown under SRC are more nutrient demanding than those growing in the wild (Weih 2001). High N, P, K, Ca and Mg contents in biomass have been measured in SRC of *Salix* (Adegbidi *et al.* 2001). Soil N availability was shown to strongly influence foliar N and aboveground yields (Ens *et al.* 2013; Toillon *et al.* 2013). Ericsson (1981b) also showed strong causal relationships between soil nutrient availability (including N, P, K, Ca and Mg), leaf nutrients and aboveground biomass yields for three *Salix* cultivars grown in hydroponics. The yields of *Salix* cultivars generally have a greater response on sandy soils with low nutrients following fertilization with wastewater sludge compared to clayey soils (Labrecque & Teodorescu 2003). This study showed that foliar N was, as a whole, improved after sludge application, whereas foliar Ca, Mg, K and P increased at some sites and remained unchanged at others. No clear link could be made between foliar nutrient responses and soil type, although coarse-textured soils tended to respond more strongly than clayey soils. Also, following fertilization of *Salix viminalis* on loamy sand soils with ammonium nitrate, municipal sewage sludge compost, municipal biocompost or willow bioash, foliar N and K were increased, while foliar P, Ca and Mg were decreased (Simon *et al.* 2013). No explanation was provided for the decrease in foliar P, Ca and Mg, but interferences with other nutrients are suspected (e.g. Ouimet *et al.* (1996) for *Acer saccharum*. Also, interactions between nutrients and other elements in the soil were also shown to

influence *Salix* foliar nutrition. For example, soils with different Ca-to-Mg ratios changes the tolerance of *Salix viminalis* growing to trace metals (Magdziak *et al.* 2011; Mleczek *et al.* 2011; Magdziak *et al.* 2013).

There are many studies monitoring inter-annual variations of foliar nutrients following fertilization of forests or plantations, but few studies have tried to establish the relationships between climatic conditions and foliar nutrition over successive years. Generally, inter-annual variations in climate appear to substantially influence *Salix* nutrition and growth. For example, Kudo (2003) observed less N in leaves of *Salix miyabeana* during warm summers compared to cooler ones. Also, Duquesnay *et al.* (2000) showed that foliar N, P, K, Ca, and Mn (but not Mg) of *Fagus sylvatica* varied over several growing seasons within the same plots. The combined inter-annual and plot effects explained more than 70% of the variance in foliar nutrition. Models were also developed in order to forecast *Salix* productivity based on seasonal climatic conditions alone (Sannervik *et al.* 2006) or in combination with soil properties (Aylott *et al.* 2008b).

Salix morphological leaf traits also change based on site nutritional and climatic conditions. In three plantations established on heterogeneous soils of northern France, leaf area of six *Salix* genotypes was highest on the site with the greatest available moisture (Toillon *et al.* 2013). Higher soil N availability and aboveground biomass yields were also associated with higher soil moisture. Similarly, fertilization with granulated municipal sludge of three SRC of *Salix discolor* and *Salix viminalis* increased leaf area, leaf mass, leaf N and aboveground yields (Labrecque & Teodorescu 2001). In Mediterranean Italy, inter-annual climatic variations and site conditions influenced leaf traits of *Fagus sylvatica L.*, *Quercus cerris L.* and *Quercus ilex L.*, which, in turn, has had an impact on aboveground productivity (Bussotti *et al.* 2000). In particular, leaf surface area, N, P, and S decreased and leaf mass area (g mm^2) increased under water stress. Leaf mass area best explained site productivity under such conditions. However, relationships among leaf nutrition (and other traits), soil

properties, climatic conditions and yields are poorly documented on an annual basis.

Using various models, we tested whether the nutritional signature of *Salix miyabeana* SX67 leaves are controlled by: (1) soil and climatic conditions across a range of sites, (2) climatic fluctuations between growing seasons, or (3) both. We also developed models that can be used to predict annual aboveground biomass yields of SX67 in southern of Quebec based on foliar chemistry and mass.

3.3 Materials and methods

3.3.1 Field sites

This study was conducted at nine SRCs of *Salix miyabeana* SX67 in southern Quebec characterized by different soil properties and climatic conditions (Table 3.1). Geographical location, site history and previous landuse, and field design of each SRC are detailed in Chapter 1 (see Table 1.1) and Chapter 2 (see Figure 2.1).

3.3.2 Estimation of annual aboveground biomass yields

At each site (or SRC), five random plots (5 × 5 m) were delineated. Using the basal area increment, annual aboveground biomass yields were estimated for each plot and site using the approach described in Chapter 1. Once cuttings are planted, a few growing seasons are needed for the stools to fully establish and reach their maximum yield potential (MYP). We therefore modeled productivity only for growing seasons that had reached MYP. We also excluded from our models the first growing seasons of subsequent rotations because they were systematically lower than MYP (Chapter 1, Figure 1.3-1.4).

3.3.3 Foliage sampling, leaf mass and nutrient analysis

Foliage was sampled in each selected plot between the last week of August and the first week of September of 2011, 2012 and 2013. However, the number of sampled sites varied between growing seasons because of the complex logistics of sampling nine sites across a large geographical range and within a short period. The number of sampled plots and stools also varied between sampling years and depended on available resources for sampling and laboratory analysis. Nevertheless, coupling the data from the different growing seasons allowed for a balanced and relatively well replicated experimental design for at least five of the nine SRCs. In 2011, four contiguous healthy stools were sampled in each of the five plots at seven of the SRCs, i.e. ABI, BOI, LAV, MTL, RXP, SJPJ and STR (140 samples). In 2012, one stool in each of the five plots was sampled at six of the SRCs, i.e. ABI, BOI, LAV, MTL, RXP and HTG (30 samples). Finally, in 2013, four contiguous healthy stools were sampled in three of the five plots at eight of the SRCs, i.e. ABI, BOI, BEL, HTG, LAV, MTL, RXP and SJPJ. In this case only, the four samples collected in each plot were bulked into one sample (24 samples). For each stool sampled in 2011, 2012 and 2013, a minimum of ten mature and healthy leaves in the upper tier of the canopy (full sunlight) were collected on the stem with the largest basal diameter.

After field sampling, leaves were oven dried at 40°C to a constant weight. For each sample, at least ten leaves were weighted before they were finely ground. A mean mass per leaf was calculated for each sample. Carbon and N concentrations were determined on the ground samples with a Vario MicroCube CHNS-O Analyzer (Elementar, Hanau, Germany) for those collected during the 2011 growing season and with a EA1108 CHNS-O Analyzer (Thermo Fisons, Waltham, MA, USA) for those collected during the 2012 and 2013 growing seasons. The ground samples were also digested with concentrated HNO_3 to determine Ca, K, Mg, Mn, Zn and Fe concentrations using atomic absorption/emission (model AA-1475, Varian, Palo Alta, CA, USA), whereas P

was analyzed colourimetrically (molybdate based method) using a QuickChem 8500 series 2 FIA System (Lachat Instruments, Loveland, CO, USA).

3.3.4 Soil sampling and analysis

Soil samples were collected from the five random plots within each SRC at a depth of 0-25 cm. Samples were air-dried and then sieved to pass through a 2 mm mesh. Soil particle size distribution was analyzed using the Horiba Partica LA-950v2 Laser Particle Analyzer (Horiba Instruments, Irvine, CA, USA). Samples from BOI, MTL and SJPJ were first pre-treated to destroy organic matter. In this respect, samples were bleached twice with NaOCl (pH = 8, 1 hour at 25°C), centrifuged (400 rpm, 15 min) and washed several times with distilled water. Samples from RXP contained more than 60% of organic matter. Thus, the bulk of organic matter was first destroyed by loss on ignition and then treated with NaOCl (as explained above) before particle size measurement. Soil pH in water was measured using a soil:solution ratio of 1:2 for mineral samples and 1:10 for organic samples. Organic and inorganic C (i.e. $CaCO_3$) were measured on all samples by loss on ignition in a muffle furnace at 575°C (15 minutes) and 1,000°C (10 minutes), respectively (Rabenhorst 1988). Total soil C and N (N_{tot}) were determined on ground samples using the EA1108 CNHS-O Analyzer. Exchangeable Ca, Mg, Mn, K, Na, Fe, Al and Zn (later referred as X_{exch}) were determined by atomic absorption/emission as indicated above after they were extracted using an unbufferred 0.1 M $BaCl_2$ solution (Hendershort *et al.* 2007b). Bulk chemical composition (SiO_2, Al_2O_3, CaO, K_2O, MgO, MnO, Na_2O, P_2O_5, Fe_2O_3, Zn, TiO_2 and Ba) of all soil samples were analyzed by X-ray fluorescence spectrometry using a Philips PW2440 system (Pananalytical, Almelo, The Netherlands) equipped with a Rhodium 60 kV end window X-ray source operating at 3 kW. The fused beads were prepared from a 1:4 soil:lithium tetra(meta)borate mixture which was heated for 18 min at 1,000°C.

3.3.5 Climatic conditions

The BioSIM model was used to simulate climatic conditions at the nine SRCs for each growing season (i.e. 2011, 2012 and 2013). Using site elevation, latitude and longitude, BioSIM uses multivariate regressions to extrapolate data from the closest climatic stations (Régnière 1996). Climatic variables were modeled from the beginning of April to the end of August using the five closest stations to each site. BioSIM yields simulated climatic data that are statistically indistinguishable from measured data (Régnière & Bolstad 1994).

3.3.6 Compositional Nutrient Diagnosis

To improve our understanding of the causal relationship between foliar nutrition and SX67 productivity, we modeled annual yields with foliar nutrients using the concentrations of foliar nutrients (CFN), which refer to mass concentrations. We also used centered logarithm ratios as a means to obtain Compositional Nutrient Diagnosis (CND) scores ((Parent & Dafir 1992), which considers the interactions between foliar nutrients. Computing CND scores imply compositional data (e.g. foliar nutrients) in a constrained space (e.g. leaf). Because not all foliar nutrients/elements (e.g. S, Al, Si and traces) are usually measured, a filling value (FV) was calculated to obtain an estimate of the proportion of the unmeasured nutrients/elements:

$$\text{Eq. 1} \qquad FV = 1 - (C + N + P + K + Ca + Mg + Mn + Fe + Zn)$$

where nutrient concentrations were expressed in mass percentages.

A geometric mean (GM) of the proportions of all foliar nutrients, including FV, was then computed as followed:

$$\text{Eq. 2} \qquad GM = (C \times N \times P \times K \times Ca \times Mg \times Mn \times Fe \times Zn \times FV)^{1/10}$$

Finally, the CND scores were determined for each nutrient with centered logarithm ratios:

Eq. 3 $$CNDscore_{Nutrient} = \ln \frac{\text{Proportion of } Nutrient}{GM}$$

where *Nutrient* refers to the nutrient or element for which the CND score is calculated. This approach removes spurious correlation between raw nutrients forming the foliage, i.e. a change in concentration of a nutrient means a change in the concentration of at least another nutrient forming the foliage (Parent & Dafir 1992). The computed CND scores are suitable to visualize the global nutritional balance of foliage with multivariate analysis, e.g. PCA (Parent *et al.* 1994b). Compositional nutrient diagnosis scores were calculated with the clr function available in the library *composition* using R 3.01 (R Core Team, 2012). For the sake of simplicity, CFN values and CND scores of C, N, P, K, Ca, Mg, Mn, Zn and Fe and leaf mass are hereafter referred to as leaf traits.

3.3.7 Data analysis

SX67 foliage was unevenly sampled 21 times throughout the three growing seasons at the nine SRCs (n = 21, i.e. 7 sites in 2011, 6 sites in 2012, and 8 sites in 2013, n = 21). This is latter referred to as the extended dataset. However, for a balanced comparison of foliar nutrition and leaf mass between growing seasons, the dataset was reduced to the data from the five SRCs for which all three growing seasons were sampled, i.e. ABI, BOI, LAV, MTL and RXP (n = 15). This is latter referred to as the reduced dataset. Finally, the dataset generated from the 2011 sampling was the only one that was large enough to build robust models across plots. This dataset uses plots means (n = 35, i.e. 5 plots × 7 sites) instead of site means (n = 7). This dataset takes into account the variations within sites and is referred to as the plot scale dataset.

It was first tested if leaf traits varied between growing seasons, independently of the conditions prevailing across the SRCs. Therefore, paired t-tests between growing seasons, i.e. 2011 vs. 2012, 2012 vs. 2013 and 2011 vs. 2013, were performed using the reduced dataset (n = 15). A permutation test was used when

the condition of normality could not be achieved (Legendre & Blanchet 2009). The level of significance of these tests was expressed with the percentage of variation, i.e. a positive value if the leaf trait increased from an older growing season to a more recent growing season, and a negative value if it decreased from an older growing season to a more recent growing season.

The extended dataset (n = 21) was also used to visualize variations in leaf traits between growing seasons within each of the nine SRCs. To do so, means of annual CND score were projected onto the first two PCA axes using the rda function available in the *vegan* library, and preserving the Euclidean distance between points. With the fanny function available in the *cluster* library, a fuzzy clustering was superimposed (Bezdek 1987). Membership values of two groups, i.e. in order to reflect the soil acid-base contrast between sites, were assigned to all objects. These values (%) were illustrated proportionally with the size of two semicircles (i.e. green and red) associated with annual samplings (Borcard *et al.* 2011).

Sites were also analyzed to determine if they caused specific foliar nutrition signatures, independently of growing seasons. For doing so, the CND scores for each growing season were used to perform a linear discriminant analysis with site as a grouping factor on the reduced dataset. Prior to this analysis, the multivariate homogeneity of variances within groups was tested using the betadisper function available in the *vegan* library. The linear discriminant analysis was then performed with the lda function available in the *MASS* package. Also, a posteriori classification was done using the predict function available in the *vegan* library.

Several linear models that were developed with the reduced and plot scale (n = 35) datasets did not respect the condition of independence. To overcome this problem, the site effect was modeled simultaneously with the explanatory variables. In this respect, a contrast of Helmert (Legendre & Legendre 2012) was performed to represent the site effect with the function contr.helmert available in the *STATS* package. Using the function varpart in the *vegan* library, the variance

of the modeled response variable was then partitioned with two sets of explanatory variables (Peres-Neto *et al.* 2006): (1) the selected variable(s) with which the causal relationship was tested (i.e. soil or leaf traits), and (2) the site effect variables (i.e. the Helmert contrast). The variance of the response variable(s) was/were therefore partitioned into three parts: (1) the effect explained only by selected variables, (2) the effect explained by selected variables and site, and (3) the effect explained only by site. The significance of the first and third partitions was tested by permutation using partial redundancy analysis (Davies & Tso 1982) with the functions rda and anova in the *vegan* library (Legendre & Legendre 2012).

The hypothesis that foliar CFN values are linearly related to soil nutrients was tested for foliar N, P, K, Ca, Mg, Mn, Fe and Zn using soil N_{tot}, P_2O_5, K_{exch}, Ca_{exch}, Mg_{exch}, Mn, Fe_{exch} and Zn_{exch}, respectively. The analysis was conducted without considering that soil chemical properties may have changed over the three years of foliar sampling. Again, the variance of annual CFN means calculated for a specific nutrient using the reduced dataset was partitioned with the respective soil exchangeable cation or oxide (according to the best R^2) as well as the site effect. Climate variables generated from BioSIM that best explained CFN means of the specific nutrient were also selected after testing them individually. Mixed models were performed with site as a random effect and climate variables as a fixed effect using the function lme in the *nlme* package. Climatic variables were also tested individually by simple linear regression using the lm function. In cases where CFN means of specific nutrients were related to both soil nutrients and climate variables, the variance was partitioned using both components. The probability that a climate variable was fortuitously collinear with a soil variable to explain CFN means was taken into account.

Linear models between foliar nutrients and annual yields were developed across the sites for individual growing seasons using both the reduced and extended datasets. These models were built from CFN values, CND scores and leaf mass,

whereas annual yield was the response variable. Each significant variable is later reported.

Partitioning of the variance of annual yields was performed with site effect and leaf traits as explanatory variables using the reduced and plot-scale datasets. We finally partitioned the variance of annual yields with site and foliage using the extended dataset to compare the percentages explained by leaf nutrients vs. leaf mass. No Helmert contrast was done because the numbers of annual measurements carried out at each site were unbalanced. The condition of independence was therefore not met, but previous analyses suggested that site effect was controlled by leaf traits (see results section). Prior to this analysis, a forward selection of explanatory variables had been performed using the forward.sel function in the *packfor* library.

Multivariate regression trees (MRT) were performed in order to explain the variance of annual yields with successive thresholds of leaf traits, e.g. leaf mass and foliar N (De'ath 2002). This non parametric method of hierarchical clustering recursively splits the response variables into two clusters whose sums of least squares are minimized. The output of MRT also provides a residual error, which yields the R^2 of the model (i.e. 1—error). Each MRT was performed with the same number of nodes (previously validated by cross validation) as significant variables used for partitioning the variance with the same data sets. These analyses were performed across sites and years using the extended and plot scale datasets. The MRTs were performed using the mvpart and MRT functions available in the *mvpart* and *MVPARTwrap* libraries. Some didactic explanations and examples are described in Legendre and Legendre (2012) and Borcard *et al.* (2011).

3.4 Results

3.4.1 Variation in soil and climate

All SRCs were established on mineral soils, except at RXP which was planted on a soil with organic C (C_{org}) exceeding 60% (Table 3.1). Soils vary considerably across the SRCs in terms of particle size distributions, with sand contents ranging from 19 to 74% and clay contents ranging from 4 to 43%. Soil chemistry also varies substantially across SRCs (Table 3.1). Soil pH ranges between 5.1 and 7.5 across the SRCs, with BOI and HTG having a more neutral pH than the others. Except for RXP which shows high N and P concentrations (9.0 mg N kg^{-1} and 1.78 mg P_2O_5 kg^{-1}), SRCs have relatively low total N (1.7-3.4 mg kg^{-1}) and P_2O_5 (0.13-0.39 mg kg^{-1}) concentrations. The STR, MTL and LAV sites have the lowest C_{org} values (<7.5%) and exchangeable Ca (<5.1 $cmol_c$ kg^{-1}) and Mg (<0.8 $cmol_c$ kg^{-1}) concentrations, and this seems to be explained by the fact that the soils of these SRCs have the highest sand contents (>50%).

Based on degree days (base 5°C) computed with the BioSIM model, the 2012 growing season was warmer than those of 2011 and 2013 (Table 3.1). Also, degree days averaged over the three growing seasons varied from 1282 to 1866 across the SRCs, with the northernmost site (ABI) showing the lowest value and one of the most southerly site (LAV) showing the highest value (Table 3.1). The amount of rainfall was the lowest during the 2012 growing season, indicating a warm and relatively dry summer. Variation in rainfall across the SRCs was not as large as for degree days. However, rainfall variations across growing seasons were higher than variations across SRCs.

Table 3.1 Average degree days (base 5°C) and rainfall for the 2011, 2012 and 2013 growing seasons and general soil properties (0-25 cm) at each of the nine SRCs. The average degree days and rainfall are also reported for each growing season and are calculated with data from all SRCs. The coefficients of variation across SRCs and growing seasons are reported in parentheses

Site	Degree days	Rainfall	Sand	loam	clay	pH	†O.M.	N_{tot}	P_2O_5	K_{exch}	Ca_{exch}	Mg_{exch}	Mn_{exch}
	—°C—	—mm—	——%——				–%–	—g Kg^{-1}—		——c_{mole} kg^{-1}——			
ABI	1282 (6%)	414 (8%)	19.4	37.5	43.1	5.64	7.9	2.4	1.3	0.98	9.6	4.0	0.047
BEL	1801 (6%)	525 (20%)	34.5	52.7	12.8	6.88	9.1	2.5	1.9	0.70	24.2	6.7	0.005
BOI	1787 (7%)	481 (19%)	34.9	46.2	18.9	7.51	9.2	3.4	2.4	1.20	17.5	5.0	0.009
HTG	1804 (5%)	502 (23%)	18.9	53.5	27.6	7.30	11.2	3.4	2.2	0.60	28.5	8.9	0.005
LAV	1866 (7%)	453 (22%)	49.6	39.1	11.3	5.27	5.4	2.0	3.9	1.71	3.1	0.2	0.016
MTL	1556 (7%)	436 (21%)	74.1	22.1	3.7	5.15	6.9	1.7	1.7	0.19	1.3	0.1	0.037
RXP	1748 (6%)	559 (26%)	46.2	48.1	5.7	5.54	67.6	9.0	17.8	0.53	63.4	2.2	0.082
SJPJ	1424 (8%)	511 (15%)	30.7	46.4	22.9	5.30	9.1	3.1	1.9	0.52	9.7	0.6	0.057
STR	1751 (6%)	532 (10%)	59.2	32.1	8.7	5.60	4.6	1.7	1.6	0.69	5.1	0.8	0.016
2011	1681 (13%)	566 (13%)											
2012	1766 (12%)	398 (11%)											
2013	1559 (12%)	496 (10%)											

SRCs belonging to the reduced dataset (n = 15) are indicated in bold. † O.M. is organic matter.

3.4.2 Variations in foliar nutrients

Concentrations of foliar nutrients varied across SRCs mostly for Mn, followed by K and Mg, then Ca and N, and finally P (Figure 3.1, Table 5.1). The CFN values and CND scores of Mg and N were among the highest at BOI, BEL and HTG and the lowest at LAV and MTL (Figure 3.2, Table 5.1). The opposite was observed for CND scores and CFN values of Mn. The RXP site (organic) was characterized by the highest CND scores and CFN values of Ca and Zn. Foliar nutritional structures were illustrated using CND scores (Figure 3.1). The fuzzy clustering highlighted a detachment of the SRCs characterized by neutral (i.e. BOI and HTG) or near-neutral (i.e. BEL) soils from the SRCs with slightly acidic to acidic soils (i.e. all other SRCs), and a further detachment of RXP and LAV from the bulk of SRCs characterized by acidic soils (Figure 3.1). The CND scores from the five SRCs belonging to the reduced dataset were also discriminated to 100% by cross validation of the linear discriminant analysis, independently of the growing seasons (Figure 3.2).

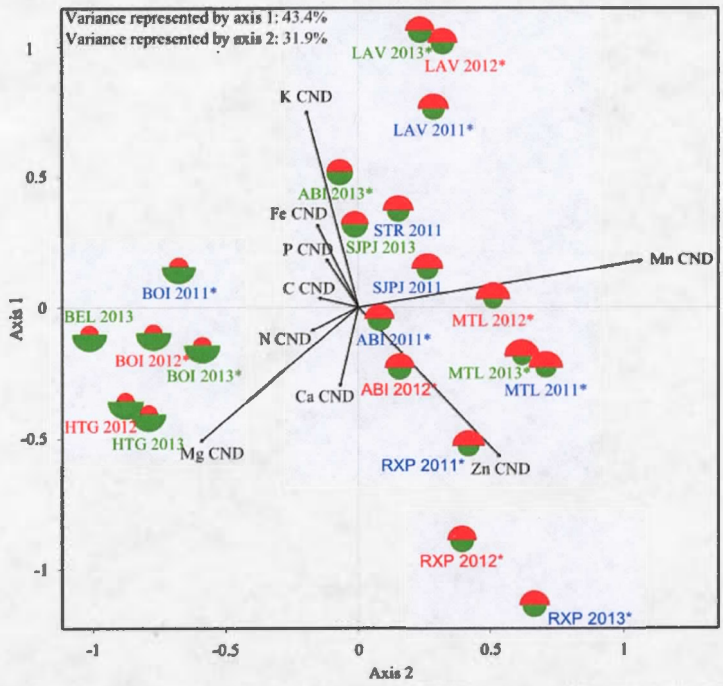

Figure 3.1 Principal component analysis of annual CND scores of C, N, P, K, Ca, Mg, Mn, Fe and Zn measured in 2011 (blue), 2012 (red) and 2013 (green) at the nine SRCs (i.e. ABI, BEL, BOI, HTG, LAV, MTL, RXP, SJPJ and STR). A fuzzy clustering is superimposed. For each object, the two clusters (i.e. red and green) equal 100% and describe the proportional similarities between two groups. The stars indicate the measurements performed on the reduced dataset (n = 15) instead of the extended dataset (n = 21).

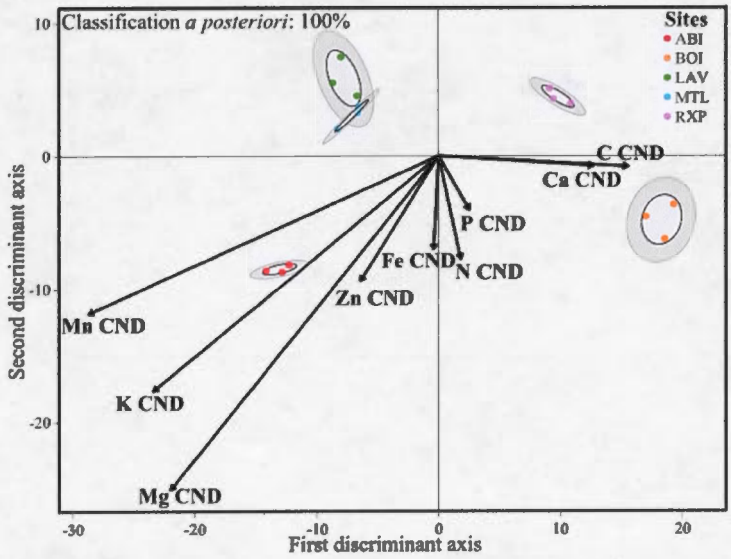

Figure 3.2 Linear discriminant analysis of CND scores of C, N, P, K, Ca, Mg, Mn, Fe and Zn by SRC (reduced dataset, n = 15). The ellipse surrounds 95% of the theoretical inner group dispersion.

Variations in foliar nutrients between growing seasons were generally lower than variations across SRCs (Table 3.2). The largest variations in CND scores between growing seasons were observed at ABI and RXP (Figure 3.1). The variation in leaf mass across growing seasons (22.9%) and SRCs (31.1%) were in the same range as those of CFN values, i.e. 13.4-31.6% and 16.7-68.6% across growing seasons and SRCs, respectively. From 2011 to 2012, foliar C, N, Ca, K and Zn decreased, whereas foliar Mg, P, Fe and Mn as well as leaf mass increased. Paired t-tests were significant for CFN values of C, N, Mg, Fe and Mn and CND scores of N, K, Fe, Ca, Mg and P. Foliar Ca, Mg and Mn decreased from 2012 to 2013, whereas foliar C, N, K, P, Fe and Zn as well as leaf mass increased. Paired t-tests were significant for CFN values of C, N and Mg, CND scores of N and Mg, and leaf mass. Finally, foliar N, Ca, K and Zn in 2013 were lower than those in 2011, whereas foliar C, Mg, P, Fe and Mn as well as leaf mass in 2013 were higher than in 2011. Paired t-tests were significant for CFN values of C, Ca, K and P, CND scores of Ca, P and N, and leaf mass (Table 2.2).

Table 3.2 Percentage of variation in CFN values and CND scores of C, N, Ca, Mg, K, P, Fe, Mn and Zn and leaf mass (LM) between growing seasons, and coefficients of variation across SRCs and growing seasons.

		C	N	P	Ca	Mg	K	Fe	Mn	Zn	LM
						%					%
2011 Vs 2012	**CFN**	-3.1**	-24.9*	+35.4	-6.6$^{(*)}$	+41.8*	-27.6*	+41.7^{*ǂ}	+21.9$^{(*)}$	-28.0^{ǂ}	+20.3
	CND	-0.7	-13.6*	+53$^{(*)}$	-3.9$^{(*)}$	+45.0$^{(*)}$	-23.8*	+5.2*	+2.5^{ǂ}	-4.7$^{(*)ǂ}$	
2012 Vs 2013	**CFN**	+6.6**	+23.5*	+5.3	-19.5^{ǂ}	-15.0$^{(*)ǂ}$	+7.6^{ǂ}	+34.9^{ǂ}	-3.7^{ǂ}	+32.7^{ǂ}	+30*
	CND	+1.2	+11.5*	+15.0	-12.9	-52.3$^{(*)}$	+3.1	+0.01	-3.2	+3.0	
2011 Vs 2013	**CFN**	+3.3*	-7.37$^{(*)}$	+42.6$^{(*)ǂ}$	-24.8*	+20.5	-22.1$^{(*)}$	+91.2^{ǂ}	+17.3	-4.5^{ǂ}	+56**
	CND	+0.4	-3.7*	+60.1*	-16.3*	+20.9	-21.4	+5.2	+2.2	-1.6^{ǂ}	
CV across SRCs		1.9	16.7	20	41.0	44.9	51.7	44.2	43.4	68.6	31.1
CV across seasons		3.2	14.3	18.1	13.4	17.3	17.5	31.6	10.2	16.9	22.9

Paired t-test results of leaf traits between growing seasons are shown as follows: $^{(*)}p\leq0.1$, $^{*}p\leq0.05$, $^{**}p\leq0.01$, $^{***}p\leq0.001$.
Growing season averages are calculated with the reduced dataset (n = 15). ^{ǂ} Condition of normality were not met.

3.4.3 Relationships among foliar nutrients, soil chemical properties and climatic variables

Soil Ca_{exch}, MnO, Mg_{exch}, K_{exch}, N_{tot} and Zn_{exch} were respectively linearly related to foliar Ca, Mn, Mg, K, N and Zn (i.e. CFN), independently of the growing seasons (Table 3.3). Site effects, which included soil effects, explained an additional (and significant) part of the variance in CFN values of K, Mn and Zn. When considering site effect as a random factor in mixed models, average air temperature, consecutive days with precipitation, radiation and total precipitation explained 94%, 81%, 93%, 92% and 90% of the variance in CFN values of C, N, Ca, K and Mg, respectively (Table 3.3). Using linear regression, only the variance in CFN values of C, N and K were significantly explained by climatic variables. Soil and climate collinearly explained a large part of the variance in CFN values of N and K (Table 3.3). The model which included the number of consecutive days with precipitation and soil N_{tot} significantly improved the prediction of the variance in CFN values of N. In contrast, soil K_{exch} explained nearly all of the variance in CFN values of K explained by radiation, and further increased the prediction of the variance in CFN values of K.

Table 3.3 Results of linear models (R^2) between CFN values (i.e. N, Ca, K, Mg, P, Mn, Fe, Zn) and their associated soil variables (i.e. N_{tot}, Ca_{exch}, K_{exch}, Mg_{exch}, P_2O_5, Mn_{exch}, Fe_{exch} and Zn_{exch}) (i.e. Soil var. column) or between CFN values and climatic variables (i.e. Clim. var. column) performed across SRCs and growing seasons (i.e. 2011, 2012 and 2013). The column entitled Site ℂ Soil var. reports the adjusted R^2 of the site effect not explained by the soil variables, whereas the column Mix. mod. reports the R^2 of mixed models of CFN values using climatic variables with site as a random factor. For climatic variables, positive (+) and negative (-) relationships are indicated in parentheses. The last three columns entitled Soil var. ℂ Clim. var., Soil var. ∩ Clim. var., and Clim. var. ℂ Soil var. indicate the adjusted R^2 of the variance of CFN values explained respectively by the soil variable only, the soil variable and climate variable, and the climate variable only, the soil variable and climate variable, and the climate variable only.

CFN	Soil variables	Soil var.	Site ℂ Soil var.	Climatic variables	Clim. var.	Mix. mod.	Soil var. ℂ Clim. var.	Soil var. ∩ Clim. var.	Clim. var. ℂ Soil var.
		adj. R^2	adj. R^2		R^2	R^2	adj. R^2	adj. R^2	adj. R^2
C	NA	NA	NA	Air temperature	0.31* (-)	0.94*** (-)	NA	NA	NA
N	N_{tot}	0.46**	0	Consecutive days with precipitation	0.52** (+)	0.81*** (+)	0.12*	0.34	0.18*
Ca	Ca_{exch}	0.82***	0	Radiation	NS	0.93* (-)	NA	NA	NA
K	K_{exch}	0.62***	0.20*	Radiation	0.46** (-)	0.92* (-)	0.23**	0.38	0.03
Mg	Mg_{exch}	0.68***	0.08	Precipitation	NS	0.90* (+)	NA	NA	NA
P	P_2O_5	NS	0.16	NS	NS	NS	NA	NA	NA
Mn	Mn_{exch}	0.26*	0.55**	NS	NS	NS	NA	NA	NA
Fe	Fe	0	0	NS	NS	NS	NA	NA	NA
Zn	Zn_{exch}	0.46**	0.30**	NS	NS	NS	NA	NA	NA

Linear models are significant at *$p \leq 0.05$, **$p \leq 0.01$ and ***$p \leq 0.001$. NA is not applicable, whereas NS is not significant. Models were developed using the reduced dataset ($n = 15$).

3.4.4 Relationships among foliar nutrients, leaf mass and aboveground biomass yields

Both the CFN values and CND scores of Ca and N were linearly related to annual yields of the 2011 growing season (Table 3.4). For the 2012 growing season, the CFN values and the CND scores of Ca were related to annual yields, whereas leaf mass was significantly related to yields only when all six sites sampled that year were considered. In 2013, CFN values and CND scores of N and leaf mass were positively related to annual yields. Also, the CND scores of Ca were positively related to yields only when all eight sites sampled that year were considered.

All of the variance in annual yields explained by the site effect was concomitantly explained by selected variables (adjusted $R^2 = 0.59$, Table 3.5). The CFN values and CND scores explained an additional 9% and 5%, respectively. Consequently, CFN values of Ca, N and Zn explained 68% of the variance in annual yields, whereas CND scores of Ca, N and Mn explained 64%.

Across SRCs, leaf mass and CFN values of Ca explained 80% of the variance in annual yields and nearly overlapped all of the site effect, whereas leaf mass and CND scores of Ca explained only 66% of the variance in annual yields and, therefore, did not totally overlap all of the site effect (Table 3.5). The CNF values and CND scores of N and Ca respectively explained 53% and 56% of the variance in annual yields. In addition, 33% and 42% of this variance explained by the combined CFN values and CND scores of N and Ca were also controlled by leaf mass. This latter variable explained another 29% and 20% of the variance in annual yields that CFN values and CND scores did not explain, respectively.

Table 3.4 Canonical redundancy analysis results (adjusted R^2) of annual aboveground biomass yields (annual yield) vs. CFN values, CND scores and leaf masses (LM) performed across SRCs for the 2011, 2012 and 2013 growing seasons individually. Positive (+) and negative (-) relationships are indicated in parentheses.

Growing seasons	N	CFN	CND	LM
2011	7	Ca 0.74** (+) N 0.63* (+)	Ca 0.75** (+) N 0.74* (+)	NS
	5	N 0.94** (+) Ca 0.82* (+)	Ca 0.84* (+)	NS
2012	6	Ca 0.57$^{(*)}$ (+)	Ca 0.80** (+)	0.70* (+)
	5	Ca 0.61$^{(*)}$ (+)	Ca 0.75* (+)	NS
2013	8	N 0.51* (+)	N 0.69** (+) Ca 0.42* (+)	0.85*** (+)
	5	N 0.80* (+)	NS	NS

Linear models are significant at $^{*}p \leq 0.05$ and $^{**}p \leq 0.01$. Models are built using either the yearly data of the reduced (n = 5 each year) or extended (n = 7 in 2011, n = 6 in 2012 and n = 8 in 2013) datasets.

Table 3.5 Partitioning of the variance of annual aboveground biomass yields between selected leaf traits (nutrients or leaf mass (LM)) and site effect. Positive (+) and negative (-) relationships are indicated in parentheses. The column entitled Sel. var. ℂ Site indicates the adjusted R^2 of the selected leaf traits which does not overlap site effect. The column entitled Sel. var. ∩ Site indicates the adjusted R^2 of the variance explained by selected variables and site effect. The column entitled Site ℂ Sel. var indicates the adjusted R^2 of site effect which does not overlap selected variables. The column entitled $R^2_{Sel.\ var}$ indicates the R^2 of the effect of selected variables as a whole. The models presented in the shaded area are similarly constructed, although they test the effect of LM instead of site effect. These models also did not meet the condition of independence.

		CFN					CND				
Year	**n**	**Selected variables**	**Sel. var. ℂ Site**	**Sel. var. ∩ Site**	**Site ℂ Sel. var.**	**$R^2_{Sel.\ var.}$**	**Selected variables**	**Sel. var. ℂ Site**	**Sel. var. ∩ Site**	**Site ℂ Sel. var.**	**$R^2_{Sel.\ var.}$**
2011	35	Ca (+) N (+) Zn (-)	0.09*	0.59	0.00	0.75**	Ca (+) N (+) Mn (-)	0.05	0.59	0.00	0.73**
2011 2012 2013	15	LM (+) Ca (+)	0.01	0.79	0.05	0.92**	Ca (+) LM (+)	0.00	0.66	0.19**	0.91**
Year	**n**	**Sel. var.**	**Sel. var. ℂ LM**	**Sel. var. ∩ LM**	**LM ℂ Sel. var.**	**$R^2_{Sel.\ var.}$**	**Sel. var.**	**Sel. var. ℂ LM**	**Sel. var. ∩ LM**	**LM ℂ Sel. var.**	**$R^2_{Sel.\ var.}$**
2011 2012 2013	21	N (+) Ca (+)	0.20**	0.33	0.29**	0.85**	N (+) Ca (+)	0.14*	0.42	0.20**	0.80**

Linear models are significant at *$p \leq 0.05$ and **$p \leq 0.01$. Simple R^2 are presented instead of adjusted R^2 in order to compare model results with those in Figure 3.5. Models are built using either the plot scale (n = 35), extended (n = 21) or reduced (n = 15) datasets.

The MRTs developed with the CFN values and CND scores as explanatory variables yielded two distinct structures (Figure 3.3). The MRT developed from the CND scores had a slightly higher R^2 – however, whether the CFN values or the CND scores were considered, the same nutrients were selected in a matching order, i.e. first Ca, then N and finally P. When site was considered instead, the CFN values and CND scores of N explained 73% and 81% of the variance in annual yields, respectively (Figure 3.5).

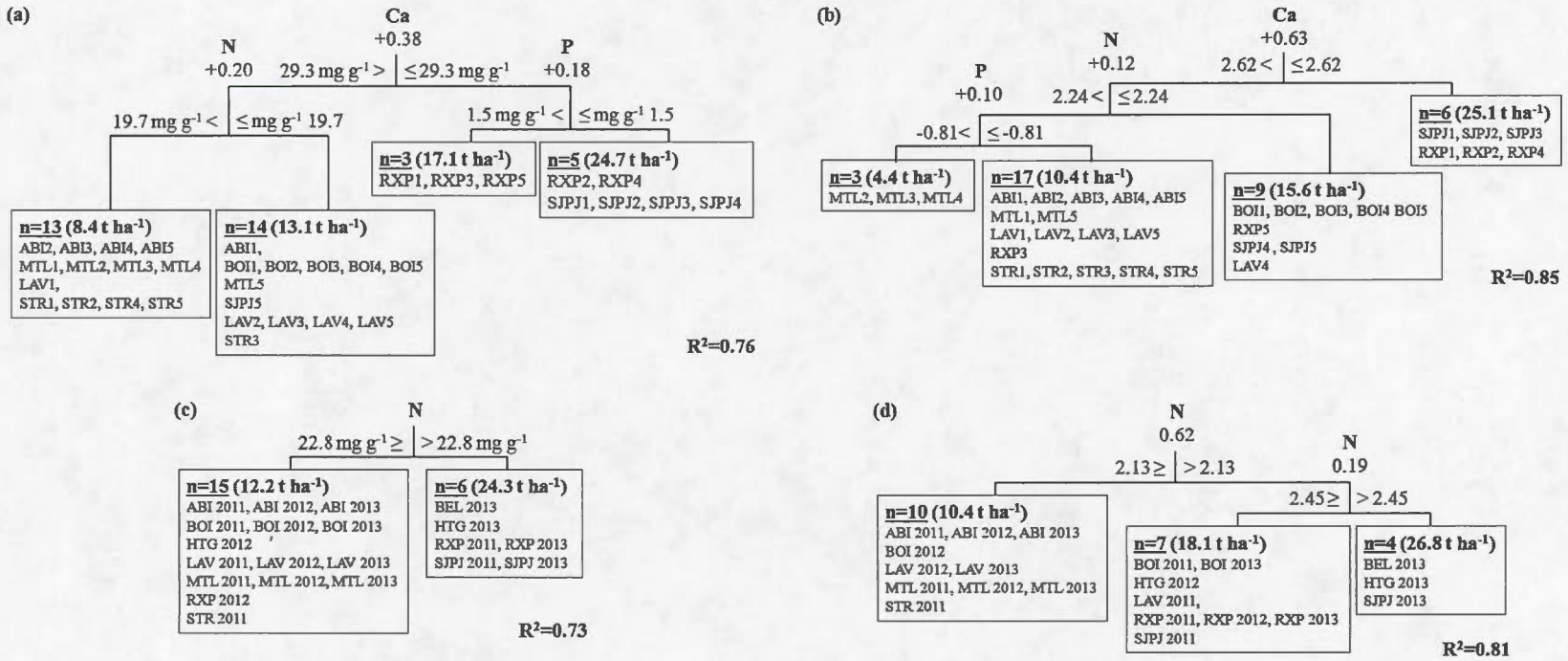

Figure 3.3 Variance of annual aboveground biomass yields explained by leaf traits through MRT using the CFN values (a) and CND scores (b) from the 2011 growing season only (plot-scale, n = 35) as well as the CFN values (c) and CND scores (d) from the 2011, 2012 and 2013 growing seasons (extended, n = 21). The R^2 (i.e. 1 – residual error) and levels of significance are presented for each node of the MRTs. For the MRTs shown in panels (a) and (b), the number following the site name (e.g. ABI1) refers to the plot number. For each cluster, the mean of annual aboveground biomass yields is reported in parentheses.

3.5 Discussion

3.5.1 Foliar signatures are controlled more largely by soil conditions than climate

In this study, we investigated foliar nutrition across a range of site conditions. In agreement with Labrecque and Teodorescu (2001), low CND scores and CFN values of N and Mg were observed at the SRCs with the sandiest soils (i.e. LAV, MTL and STR, Figure 3.1 and Table 5.1). As a whole, soil pH values also appeared to have an influence on the balance of nutrients in the leaves. For example, the SRCs with the more neutral soils (i.e. BEL, BOI and HTG) had a relatively similar status in foliar nutrients (Figure 3.1).

For *Fagus sylvatica* in northeastern France, Duquesnay *et al.* (2000) reported that the spatial variability of CFN values was low for C, slightly variable for N and P, moderately variable for K and Ca, and highly variable for Mn and Mg. Based on the coefficients of variation calculated to describe differences in CFN values across the studied SRCs of *Salix miyabeana* SX67 in southern Quebec (Table 3.2), it can be concluded that nutrients follow a similar sequence, except that K, Ca, Mg and Mn can be pooled into one group with similar coefficients of variation. Interestingly, C was the only leaf element for which the variation between growing seasons was higher than those between SRCs (Table 3.2).

We also observed two additional trends for CFN values. For one, variations in CFN values were smaller between growing seasons than across SRCs for Ca, Mg, K, Mn and Zn. Second, variations in CFN values between the growing seasons for N, P and Fe were quite comparable to those across SRCs (Table 3.2). We therefore tested if specific foliar nutritional signatures could be distinguished for the various SRCs, independently of the variations between growing seasons. Discriminating the nutritional signatures based on site was highly efficient, independently of the growing seasons (see length of vectors in Figure 3.2). Compositional nutrient diagnosis scores are generally well suited for multivariate analysis (Parent *et al.* 1994a). This seems to be verified with our dataset because, using that same statistical approach, we failed to discriminate site-specific foliar

nutritional signatures with CFN values alone (results not shown). Spurious correlations of CFN values can generate interpretation problems when using multivariate analysis and can be removed by log-ratio transformations which provide variables that are linearly independent (Parent *et al.* 2013).

Across SRCs and growing seasons, we found relationships between major foliar nutrients and associated soil nutrients for all nutrients except P (Table 3.3). It is possible that P_2O_5 was not the best indicator of soil P availability, but the relationships between soil and foliar P has not always been straightforward (Ling & Silberbush 2002; Bowman *et al.* 2003; Ens *et al.* 2013). The correspondence between soil and foliar nutrients other than P have been reported for various tree species, including deciduous species (e.g. Afif-Khouri *et al.* (2011) for *Castanea Sativa* in Spain; Orgeas *et al.* (2003) for *Quercus suber*) and *Salix purpurea* (Ens *et al.* 2013). Ericsson (1981b) suggested a mechanism of diffusion for the uptake of Ca and Mg by *Salix* grown in hydroponics. Similarly, CFN values of Ca, K and Mg were depicted for *Pinus abies* and *Fagus sylvatica* foliage sampled from Italy to Norway (Bauer *et al.* 1997). They found foliage to be a relatively robust mirror of soil Ca, K and Mg availability. Soil Mn availability is negatively related to its pH (Sanders 1983; Martínez & Motto 2000), and higher CND scores of Mn were observed at the SRCs characterized by lower soil pH (Figure 3.1, Table 3.1 and Table 5.1). In addition, a strong relationship was observed between CFN values of Mn and soil pH (adj. $R^2 = 0.80$, results not shown) which perfectly overlapped site effect (Table 3.3) and also suggested a diffusive path for Mn.

In a study conducted across Canada, Ens *et al.* (2013) observed a strong linear relationship ($R^2 = 0.85$, $p<0.05$) between CFN values of N and soil N_{tot}. The data collected in our study produced a similar but weaker relationship (Table 3.3), perhaps because the dataset included inter-annual variations in CFN values of N which were specific to each growing season (Table 3.2). Independently of soil conditions, only CFN values of N (among all leaf nutrients measured) were significantly explained by a climate variable (Table 3.3). Our results are consistent with a previous study showing that N uptake of *Salix* was lower under conditions

of water stress (Bowman & Conant 1994). Christersson (1986) emphasized the relationship between soil water content and foliar N levels of *Salix dasyclados* and *viminalis* — foliar N decreased in the following order: irrigation in combination with fertilization > fertilization without irrigation > neither irrigation nor fertilization (or control).

Climate had a lesser influence on foliar nutrition compared to soil. This was also observed for *Salix purpurea* in studies conducted from central to eastern Canada (Ens *et al.* 2013) or at the global scale (Ordoñez *et al.* 2009). For example, the study by Ordoñez *et al.* (2009) showed that total soil N, P and C:N ratio were much better predictors of the variance in leaf traits (i.e. specific leaf surface area, foliar N and P) of 474 species across 99 sites compared to climatic variables (i.e. precipitation, temperature, irradiance and evapotranspiration). Nevertheless, multivariate analysis revealed that predictions of leaf traits, including N and P, were improved when interactions between soils and climate were considered. Thus, using mixed models with site as a random effect allows investigating the influence of climatic variables, regardless of site specificity. Across 42 locations in Australia, for example, mixed models highlighted that the combined effects of site and temperature strongly influenced foliar N and secondary metabolites of *Eucalyptus microcorys* (Moore *et al.* 2004). In our study, mixed model analysis systematically improved the strength of the relationships compared to simple linear regression (Table 3.3). A strong relationship between CFN values of Ca and total radiation emerged with a mixed model. It should be noted that our study was not built to identify causal mechanisms. However, Kobe (1996) suggested that CFN values of Ca improved light use efficiency and tolerance to low light availability by *Fagus grandifolia*, *Fraxinus americana* and *Acer saccharum*.

3.5.2 Foliar traits impact annual aboveground biomass yields

Because foliar nutrient levels changed between growing seasons, nutrients impacting annual yields also differed between growing seasons (Table 3.4). The

positive relationship between foliar N levels and yields observed by Ens *et al.* (2013) and Ericsson (1981a) for *Salix* spp. was also observed in our study for SX67 in 2011 and 2013, but not in 2012. The 2012 growing season was the driest of the three years (Table 3.1), which could mean that water availability was restricting growth. Optimal foliar nutrients of tree species was reported to be affected by drought in the past, mainly for macronutrients such as N and P (Vizcayno-Soto & Côté 2004). The filling value in Equation 1 was higher for the 2012 growing season, meaning a lower acquisition of nutrients by SX67. However, Weih *et al.* (2011) studied 200 *Salix* genotypes and found that those with higher CFN values of N were best adapted to withstand water shortages. Under such conditions, the growth of *Salix genotypes* with higher foliar N reserves was more impeded by photosynthetic limitations due to drought than due to a concomitant decrease in foliar N. In our study, water stress due to low rainfall in 2012 varied as a function of intrinsic soil properties (e.g. physical variables such as depth, porosity and particle size distribution) and thus, it likely modulated the magnitude of photosynthetic limitations differently at each SRC.

In 2011 and 2012, the CFN values and CND scores of Ca were positively related to annual yields (Table 3.4). For *Salix purpurea*, Ens *et al.* (2013) found a linear relationship between biomass yields and total soil Ca levels across a field design that covered a wider spectrum of soil Ca availability (felsic (acid) to carbonate (neutral) soils) compared to our study. These authors, however, did not find a relationship between CFN values of Ca and total soil Ca levels, nor between CFN values of Ca and biomass yields. They concluded that total soil Ca was probably a better reflection of the impact of soil acidity on *Salix purpurea* growth than the impact of Ca availability on growth *per se*. Moreover, positive linear relationships between basal area increment and foliar Ca of *Quercus rubra* and *Pinus strobus* were shown for five locations (northern New York to western Maine) characterized by sandy acidic soils, whereas foliar N was shown to have a small impact on yields only for *Pinus strobus* (Hallett & Hornbeck 1997). The availability of Ca is also well known to limit the growth of *Acer saccharum* on

acidic soils (St.Clair *et al.* 2008) and liming has both beneficial outcomes on foliar Ca and growth (Horsley *et al.* 2000; Juice *et al.* 2006). We cannot easily explain the fact that Ca was not linked to willow productivity during the most productive growing season. However, it should be noted that the degree-days were higher for 2011 and 2012 (Table 3.1) which might have favored greater transpiration rates. Because Ca uptake is one of the few nutrients controlled by diffusion from the soil solution (Ericsson 1981b), it is possible that foliar Ca levels were a strong fingerprint of soil Ca_{exch} (adj. $R^2 = 0.82$, Table 3.3).

At the plot scale, the annual yield models developed from CFN values and CND scores had lower adjusted R^2 (Table 3.5). In this case, however, the variability within plots was taken into account. These models showed that: (1) site effect was completely controlled by the selected leaf traits, (2) variability within plots was not well explained, indicating that variation in annual yields within the sites was mainly influenced by nutritional factors specific to each site, and (3) CND scores of Mn in 2011 were negatively linked to annual yields, whereas those of Ca and N were positively related to annual yields.

Our study was conducted on soils that are generally acidic (Table 3.1). As a result, soil Mn availability was likely increased under such conditions. Manganese activity in the soil solution is further increased under reducing conditions (Gotoh & Patrick 1972; Sanders 1983). *Salix* can survive under relatively high CFN value of Mn (Yang *et al.* 2015), whereas toxicity symptoms with reduced growth mostly appear in poorly drained conditions (Kovalchik 1992). A negative impact of Mn on yields has also been reported for hybrid poplar in central Alberta (Pinno *et al.* 2010) and *Acer saccharum* growing on acidic soils in southern Quebec (Houle *et al.* 2007). In our study, the negative impact of Mn on growth appeared only when using the in 2011 data (plot scale analysis). The CFN values of Mn were rather homogeneous across the growing seasons, suggesting that the negative effect of Mn on SX67 was masked by more important factors restricting annual yields (i.e. N and Ca) when analysing at the landscape scale. Across SRCs and growing seasons, leaf mass was the most robust predictor of annual yields (Table 3.5).

Such an observation was also made by Weih *et al.* (2006) in Sweden. In our study, leaf mass overlapped a large part of the effects of foliar nutrients on annual yields and explained an additional 20% and 29% of its variation using CFN values and CND score, respectively. This suggests a proportional investment of C for leaves and wood when analyzing at the landscape scale. However, this was not observed when using the plot data for the 2011 growing season (Table 3.4 and Figure 3.3).

Using MRT, a threshold of 22.8 mg N g^{-1} was identified for CFN values of N, beyond which no annual yield smaller than 20 Mg ha^{-1} yr^{-1} was observed (Figure 3.3c). The threshold suggested by Kopinga and Van den Burg (1995) for *Salix viminalis* (i.e. <20 mg N g^{-1} means a deficiency, 23-30 mg N g^{-1} is normal >30 mg N g^{-1} is optimal) were higher than our observations. The observed CFN values of N for SX67 were also lower than for *Salix viminalis* and *discolor* after sludge application (Labrecque *et al.* 1998). However, they were in a range of values previously reported for other *Salix* cultivars (Viherä-Aarnio 1994; Ens *et al.* 2013). Furthermore, SX67 yields at some of our sites (i.e. BEL, HTG and SJPJ) were among the highest values reported for *Salix* cultivars grown in southern Quebec (Labrecque & Teodorescu 2005; Guidi Nissim *et al.* 2013) or other similar temperate climates around the world, e.g. in Poland (Szczukowski *et al.* 2002). These findings suggest that SX67 uses N very efficiently to produce biomass compared to other *Salix* cultivars.

Moreover, CFN values of Ca for SX67 (i.e. 17-37 mg g^{-1}) were dramatically greater than those reported for other *Salix* cultivars (i.e. 7-19 mg g^{-1}) supported by various soil types (Viherä-Aarnio 1994; Labrecque *et al.* 1998; Simon *et al.* 2013). This suggests a high requirement for SX67. This is especially striking considering that fast growing species such as *Salix* or *Populus* spp. are already known for holding large reserves of Ca in their foliage (Ericsson 1981b; Camiré & Brazeau 1998). In particular, foliar Ca is known to increase physical protection against diseases (Franceschi 2001). Our foliar Ca results may provide some insight as to why *Salix viminalis* is more sensitive to insect infestation in southern Quebec than SX 67 (Labrecque & Teodorescu 2005).

3.5.3 Model performance to predict annual aboveground biomass yields is high

In this study, the MRTs were more suitable than linear models to explain annual yields and to detect variations at the plot scale and across SRCs and growing seasons (Table 3.5). In particular, using CND scores of each plot for the 2011 growing season, we were able to isolate the three most nutritionally deficient and least productive MTL plots using the MRT approach (Figure 3.3b). Also, at the landscape scale, MRT showed a cluster of the three SRCs with the greatest annual yields for a given growing season, i.e. BEL, HTG and SJPJ in 2013 (Figure 3.3d). The few studies that used regression trees to model tree growth from foliar nutrients have all reinforced the idea that it is a highly relevant and efficient approach (Afif-Khouri *et al.* 2011; Álvarez-Álvarez *et al.* 2011; Amichev *et al.* 2011; Ouimet *et al.* 2013a).

Major foliar nutrients that exhibited a linear relationship with annual yields of SX67 when analyzing the plot data (i.e. N and Ca) also appeared in the MRTs, whereas only foliar N was detected by the MRT when analyzing the data across SRCs and growing seasons. This is in agreement with the results of Afif-Khouri *et al.* (2011) and Álvarez-Álvarez *et al.* (2011) who compared the selected leaf traits by linear models and by regression trees to predict the growth of *Pinus pinaster* and *Castanea sativa* in Spain. They found a strong correspondence between the two methods in regard to selected variables. In both studies, the linear relationships between growth and foliar P were significant; however, the regression trees did not detect critical leaf nutrient or mass thresholds. In contrast, Ouimet *et al.* (2013b) diagnosed P deficiency for *Acer saccharum* in southern Quebec with regression trees. In our study, CFN values and CND scores of P were significant variables in the MRTs when analyzing the plot data, but they were not linearly related to SX67 productivity. The study plots with CFN values above 1.5 mg P g^{-1} and 29.3 mg Ca g^{-1} were the most productive (Figure 4.3a). Kopinga and Van den Burg (1995) reported that foliar P concentrations above 2.1 mg g^{-1} were

optimal for *Salix viminalis*. Again, our results suggest that SX67 has a better P use efficiency than *Salix viminalis*.

The MRTs that were built with CND scores, had higher R^2 than those built with CFN values (Table 3.5 and Figure 3.3) dataset. In southwestern Quebec, the global imbalance index calculated with the entire CND scores indicated that lower aboveground biomass yields of hybrid poplar were linked to nutritional imbalances (Lteif *et al.* 2008). The CND, which considers the nutritional structure of foliage, is a suitable and unbiased approach to understand plant nutrition, especially when combined with principal composition analysis (Parent *et al.* 1994b). It also allows the detection of nutrient interactions (Raghupathi *et al.* 2002; García-Hernández *et al.* 2004). Our MRT results emphasize the need to look at foliar nutrition as a whole instead of considering nutrients individually (Parent & Dafir 1992).

3.6 Conclusion

In this study, climate and site effects on foliar nutrition of *Salix miyabeana* SX67 were investigated. Despite climatic variations across growing seasons, specific foliar signatures were largely imposed by the soil chemical footprint of the site. Only foliar N and P varied as much across the years as across the sites. As a whole, during moist conditions (assuming no large water stress), N was the most important nutrient limiting annual aboveground biomass yields of SX67 in Quebec, followed by Ca. Manganese had a negative impact on SX67 yields. It was possible to use leaf traits to express the synergy between soil and climate that acted upon SX67 foliar nutrition and annual yields. However, linear models encompassing several growing seasons were not sufficiently robust to forecast yields accurately. A threshold approach of foliar nutrients allowed to: (1) deal with the variation across growing seasons and sites and (2) forecast yields successfully. The possibility to build models that warrant a minimal productivity for a given leaf nutrient concentration (in our case N) or mass was also validated.

CONCLUSIONS GÉNÉRALES

4.1 Potentiel de productivité et effet du recépage

De nombreuses CCR de *Salix* sont établies au sein du Québec méridional. Ce travail confirme que le cultivar *Salix miyabeana* SX67 est parfaitement adapté à cette région. En effet, des rendements avoisinant les 30 t ha^{-1} an^{-1} ont été mesurés (chapitre 1), ce qui est supérieur aux rendements les plus élevés observés dans cette même région avec *Salix viminalis*, lequel est un cultivar également considéré comme très productif (Labrecque & Teodorescu 2003). Un des défis de cette étude était de comparer les productivités de CCR ayant des tiges et des systèmes racinaires d'âges différents. L'utilisation de la dendrochronologie a permis de surmonter ce problème en retraçant les productivités annuelles passées, ce qui a aussi permis de déterminer un potentiel maximal de productivité. Usuellement, la productivité de *Salix* est estimée à l'aide de la masse sèche des tiges en fin de cycle, donnant ainsi accès à une information intégratrice de plusieurs saisons de croissance (Moukoumi *et al.* 2012; Ens *et al.* 2013; Toillon *et al.* 2013). De ce fait, l'usage de la dendrochronologie a permis d'enrichir l'information obtenue avec les mesures annuelles de biomasse *in situ*. Le suivi de la dynamique de croissance annuelle est une originalité de ce travail qui a permis de mettre en exergue les années où les faibles rendements observés étaient dus aux systèmes racinaires encore mal établis ou aux conditions climatiques défavorables. Comme fréquemment observé (Verwijst 1996; Volk *et al.* 2011), notre étude confirme que la première année de productivité est toujours plus faible que les suivantes, y compris au cours des deuxièmes révolutions pour lesquelles cet effet est moins marqué compte tenu des réserves de carbohydrates accumulées dans les racines (Von Fircks & Sennerby-Forsse 1998). Cette plus faible productivité est due, entre autre, à la demande en carbone nécessaire à la formation de nouvelles tiges dont le bilan énergétique devient positif seulement lorsque les premières feuilles se développent.

Sans contrainte mécanique majeure imposée à l'appareil racinaire et dans des sols n'ayant pas de teneurs nutritionnelles trop élevées (sols sableux), le potentiel de productivité peut-être à son maximum à partir de la deuxième saison de croissance. En revanche, quand le système racinaire doit faire face à des contraintes physiques importantes comme dans des sols compactés à fortes teneurs en argile, quatre saisons de croissance peuvent être nécessaires avant que la croissance soit optimale (chapitre 1). Cet écart est similaire à celui rapporté par Kopp *et al.* (2001) pour différentes conditions de fertilisation, hormis que ces auteurs ont observé que le rendement maximal suite à la fertilisation n'était pas plus élevé, mais le délai pour l'atteindre était plus court. Quoi qu'il en soit, le laps de temps requis pour une production épigée maximale est directement relatif à la différence de productivité des deux premières révolutions (chapitre 1). Le recépage effectué après la première année d'établissement de la CCR (recépage initial) semble raccourcir ce laps de temps que d'une seule année. On peut donc penser que le rendement d'une révolution après recépage initial ne soit pas plus haut (voir plus faible) que le rendement d'une première révolution qui n'aurait pas subi de recépage initial parce que cette dernière aurait bénéficié d'une saison de croissance supplémentaire. De plus, toute pratique visant à minimiser le nombre de recépage permettrait d'optimiser le bilan en carbone des CCR. À noter qu'à la CCR de LAV à la fin de sept saisons de croissance de la première révolution ou à la CCR de HTG à la fin de quatre saisons de la quatrième révolution, la productivité annuelle n'a pas décliné. Ces observations corroborent les rares études ayant suivi les rendements sur plusieurs révolutions (Quaye & Volk 2011; Guidi Nissim *et al.* 2013). Par conséquent, il sera fort instructif de faire le suivi du rendement de ces sites au cours des prochaines révolutions.

4.2 Les limitations nutritionnelles inter-sites

L'azote est largement reconnu comme un facteur qui limite la croissance de *Salix* (Ericsson 1981a; Labrecque *et al.* 1998; Weih & Nordh 2005; Quaye & Volk

2011). Dans cette thèse, des relations ont été observées entre le N foliaire et le rendement entre les sites (chapitre 3). Les CCR étudiées ici ont été établies sur des sols dont les pH variaient de 5,1 à 7,5. Sous de telles conditions, les teneurs en Ca_{exch}, en Mg_{exch} et les valeurs de pH des sols étaient positivement liées à la productivité inter-site (chapitre 2). Par conséquent, le Ca foliaire (chapitre 3) ainsi que le Mg foliaire étaient aussi liés à la productivité, mais cela dans une moindre mesure. De façon similaire, dans des sols acides, le Ca foliaire a déjà été limitant pour des espèces d'arbre comme *Quercus rubra*, *Pinus strobus* ou encore *Acer saccharum* (Hallett & Hornbeck 1997; St.Clair *et al.* 2008). Aussi, le Mn foliaire était négativement lié à la croissance de *Salix miyabeana* SX67, mais n'était limitant que dans une moindre mesure comparativement à N (chapitre 3). À noter que la toxicité du Mn sur la croissance des feuillus est fréquente sur sols acides (Houle *et al.* 2007; Pinno *et al.* 2010).

4.3 Les limitations hydriques

Une limitation de la croissance de *Salix* due aux faibles précipitations avait déjà été suspectée dans le sud du Québec (Labrecque & Teodorescu 2005). Aussi, au sein des sites étudiés dans cette thèse, la sécheresse a significativement limité la croissance, hormis au site de LAV où la productivité était positivement liée à la sécheresse, probablement parce que ce site ne subit pas de carence en eau (D'Arrigo *et al.* 2008). Cette hypothèse est corroborée par le fait que le taux de fractionnement du ^{13}C au site de LAV était parmi les plus élevés en 2011 (chapitre 2). À l'opposé, au site de ABI, le confinement des racines dans l'horizon Ap (voir Figure 2.7) a induit d'importants stress hydriques qui se sont traduits par un fractionnement du ^{13}C beaucoup plus faible qu'aux autres sites. Néanmoins, au sein de notre aire d'étude, la pluviométrie est relativement homogène et n'a donc faiblement expliqué le potentiel de rendement au sein des sites ($\approx$ 10 à 20%) comparativement aux propriétés pédologiques ($\approx$70%). En revanche, les variations des précipitations interannuelles étaient significatives et ont engendré des écarts

de productivité allant jusqu'à 44% au sein du même site. Toutefois, ces variations restent bien inférieures à l'écart maximal de productivité observé entre deux sites, soit 380%.

4.4 Les spécificités des sites

À l'instar des observations faites par Pinno *et al.* (2010), les variables pédologiques modulant la productivité étaient spécifiques à chaque site (chapitre 2). Par exemple, au site de LAV, les fortes teneurs en P total dans le sol ont induit une nutrition de luxe et généré un indice CND de P négativement corrélé avec le CND de N et la productivité de *Salix*. Aussi, la CCR de SJPJ est établie le long d'une légère pente. Le sol est aussi parmi les plus acides des 10 CCR, très caillouteux et drainant. Ces conditions de pente ont probablement favorisé la formation d'un gradient de pH inter-placettes (avec le pH le plus acide en amont). De plus, les variations de productivité intra-sites étaient fortement liées à l'indice CND de Ca ainsi qu'au Ca total.

Un des aspects novateur de cette thèse est d'avoir mis en évidence une signature nutritionnelle foliaire propre à chaque site, indépendamment des fluctuations annuelles (chapitre 3). Mais ceci a pu être démontré qu'avec les indices foliaires CND, lesquels sont linéairement indépendants. En prenant en considération la teneur du nutriment relativement à la balance nutritionnelle globale, ces indices sont parfaitement adaptés aux analyses multivariées (Parent & Dafir 1992; Parent *et al.* 1994a). Également, les variations interannuelles de N et de P étaient comparables aux variations inter-sites, tandis que celles de K, Ca, Mg, Mn, Zn et Fe étaient jusqu'à quatre fois plus importantes que les variations annuelles (chapitre 3). Par conséquent, ces derniers ont marqué plus fortement la signature nutritionnelle foliaire de chaque site.

4.5 Variations interannuelles des nutriments foliaires

Du fait de cette spécificité des sites, des modèles mixtes ont été utilisés et ont permis de montrer que les teneurs de certains nutriments foliaires (N, K, Ca et Mg) dépendaient directement des conditions climatiques, relativement aux caractéristiques intrinsèques du site (prises en considération par l'effet aléatoire). Hormis pour le N, très peu d'études ont été effectuées sur les relations entre les variations interannuelles des nutriments foliaires et les variables climatiques. Dans cette étude, la teneur foliaire en Ca était négativement liée aux radiations annuelles (chapitre 3). Ceci peut être mis en relation avec les observations faites sur *Fagus grandifolia*, *Fraxinus americana* et *Acer saccharum*, dont la hausse des teneurs en Ca foliaire augmente la tolérance aux conditions pauvres en lumière en augmentant l'efficience de son utilisation (Kobe 1996).

Par conséquent, les facteurs nutritionnels limitant la productivité inter-site ont été différents selon les années (chapitre 3). Par exemple, les teneurs foliaires en N ont varié significativement au cours des saisons de croissance selon le nombre de jours de précipitation. Lorsque les conditions climatiques nécessaires à une productivité annuelle optimale étaient réunies, la masse foliaire et le N ont été fortement liés à la productivité inter-site. En revanche, la saison de croissance de 2012 a été caractérisée par des périodes de sécheresse marquées. De ce fait, le manque d'eau semble avoir été plus limitant que la nutrition, cette dernière ne semblait alors plus liée à la productivité inter-site.

Afin de passer outre ces variations annuelles, l'arbre de régression multivarié s'est avéré être un outil de choix. Par exemple, les quatre plus forts rendements (enregistrés au cours de l'année 2013) sur les quatre sites les plus productifs ont pu être mis en évidence car tous avaient des teneurs supérieures au seuil de 24 mg de N par g de feuille (chapitre 3). De plus, bien qu'aucune relation linéaire n'ait été détectée entre le P foliaire et la productivité de *Salix*, cette méthode non-paramétrique a permis de définir des seuils nutritionnels foliaires qui suggèrent une suffisance en P. Il faut également souligner que les arbres de régression

multivariés ont expliqué systématiquement une plus forte proportion de variance que les relations linéaires, confirmant ainsi la pertinence d'apprécier le statut nutritionnel selon une approche impliquant des seuils (Ouimet *et al.* 2013a).

L'un des points forts de cette étude est que l'information nutritionnelle contenue dans les feuilles, laquelle intègre toute la saison de croissance, a pu être directement associée à la productivité de l'année considérée. En étudiant les interactions entre le climat, la nutrition foliaire et la productivité au cours de trois saisons de croissance sur diverses stations soumises à des conditions pédoclimatiques contrastées, cette étude fait figure de pionnière. En effet, à ma connaissance, aucune étude sur la productivité des arbres et des arbustes ne s'est appliquée à étudier simultanément les interactions entre les variations annuelles de ces différents facteurs.

4.6 Retombées pratiques

Le but pratique de cette thèse de doctorat était de fournir des outils de diagnostic simples pour les exploitants de *Salix miyabeana* SX67 afin qu'ils puissent estimer sa productivité *in situ*. La surface et le N foliaire ont été décrits comme de très bons indicateurs pour différents cultivars cultivés en pots (Weih & Nordh 2005). Dans cette thèse, des équations capables de garantir une productivité annuelle minimale à l'aide des teneurs en N foliaire ou avec la masse foliaire ont été établies. De plus, au-delà des seuils de 82 mg feuille^{-1}, 156 mg feuille^{-1} et 21.4 mg N g^{-1}, des productivités supérieures à, respectivement, 10 t ha^{-1} an^{-1}, 20 t ha^{-1} an^{-1} et 14 t ha^{-1} an^{-1} sont attendues.

Également, un modèle pédoclimatique a été développé dans le but d'estimer la productivité maximale d'un site en fonction de la teneur en limon et du nombre de jour de sécheresse. Cette thèse démontre l'impact prépondérant du sol sur la productivité comparativement à l'effet du climat ; avec une simple analyse granulométrique de la teneur en limon de ses terres, un exploitant peut déjà avoir une très bonne idée du potentiel de productivité de SX67.

4.7 Axes de recherche à développer

Cette thèse a permis de faire avancer l'état des connaissances, mais a également soulevé un certain nombre de problématiques qui mériteraient, à mon sens, d'être creusées davantage.

Tout d'abord, avant l'établissement d'une CCR, un labour est communément effectué en combinaison avec l'application d'un herbicide afin de mieux contrôler la compétition (Guidi *et al.* 2013). Toutefois, compte tenu de son impact sur la structure du sol (confinement des racines au-dessus de la semelle de labour, diminution de la capacité au champ, etc.) et sur les nutriments (minéralisation du C_{org}, lessivage, etc.), les résultats de mes études remettent en question l'effet positif du labour sur la croissance de *Salix*. Cependant, ces effets n'ont jamais été quantifiés. L'étude des conséquences du labour sur la productivité de CCR de *Salix* établies sur différents types de sol me parait des plus pertinente, particulièrement pour des zones comme le Québec méridional qui connaissent des limitations hydriques.

De plus, bien que l'on récolte usuellement les tiges après 3 à 5 ans, aucune étude n'a rapporté, à ma connaissance, une diminution de croissance en fin de révolution. Ainsi, il semblerait utile de mener des études sur différents cultivars pour déterminer l'âge maximal à partir duquel la productivité annuelle décline. De façon similaire, à ma connaissance, aucune limite sur le nombre de révolutions que l'on peut effectuer sur une CCR sans perdre de rendement n'a été clairement déterminée jusqu'à maintenant.

Comme pour toute culture, suite à la récolte d'une CCR de *Salix*, des quantités non négligeables de nutriments sont exportées. Celles-ci dépendent du type de sol et de la productivité du site (Hangs *et al.* 2014b). Ceci laisse néanmoins penser que les rendements devraient diminuer suite aux récoltes successives. Cependant, dans cette thèse et comme Guidi Nissim *et al.* (2013) et Volk *et al.* (2011) l'ont déjà observé (Volk *et al.* 2011; Guidi Nissim *et al.* 2013), aucune baisse des

rendements est observable pour les troisième et quatrième révolutions. On note plutôt des augmentations dans les rendements. Ceci peut-être en partie imputable à la fertilisation effectuée en théorie après chaque recépage (au moins 100 Kg N). Néanmoins, cette recommandation n'a pas été suivie dans la majorité des plantations utilisées dans cette étude cas. On peut ainsi suspecter des mécanismes capables de contrebalancer les exportations de nutriments suite aux récoltes. Ainsi, la minéralisation de la litière (Hangs *et al.* 2014a) ainsi que l'altération des minéraux par l'action des racines (Calvaruso *et al.* 2006; Lafleur *et al.* 2013) sont des phénomènes connus. De plus, les CCR sont reconnues pour augmenter la teneur en matière organique dans le sol (Zan *et al.* 2001; Routhier *et al.* 2014; Lafleur *et al.* 2015). Cependant, à ma connaissance, aucune étude ne s'est intéressée à quantifier l'effet de cette augmentation sur les propriétés du sol (CEC, propriétés hydro-structurales, activité biologique, etc.) susceptibles d'optimiser la productivité.

De plus, l'utilisation d'engrais verts (Cherr *et al.* 2006) et la culture intercalaire avec des légumineuses (Moukoumi *et al.* 2012; Moukoumi *et al.* 2013) permettraient de valoriser la surface de terre cultivée, autant sur le plan des propriétés pédologiques que sur le plan économique. Ainsi, étudier les bilans nutritionnels de cultures associées soumises à des conditions pédologiques hétérogènes permettrait possiblement d'optimiser les pratiques de culture et de fertilisation.

Enfin, cette thèse a démontré que les teneurs d'un certain nombre de nutriments foliaires varient d'une année à l'autre de façon similaire sur tous les sites, comme par exemple le N qui dépend des précipitations (chapitre 3). Des variations significatives ont été observées concernant la majorité des nutriments foliaires. Elles n'ont cependant pas été mises en relation avec les données climatiques. Ainsi, les facteurs modulant la variation interannuelle de nutriments foliaires (hormis l'N) restent à identifier.

Les résultats publiés dans cette thèse suggèrent que la promotion de *Salix miyabeana* SX67 comme cultivar pour l'établissement de CCR dans le sud du Québec est une bonne stratégie, comme ailleurs dans les régions avec un climat tempéré, parce qu'il a de bonnes capacités d'adaptation et permet des rendements élevés. Néanmoins, cette thèse suggère également de changer certaines pratiques couramment effectuées, bien que des études supplémentaires soient nécessaires pour confirmer complètement cette hypothèse.

Compte tenu de l'investissement énergétique requit par la récolte et des dégâts potentiels pouvant être engendrés par la machinerie (compaction et écrasement des racines), il semblerait raisonnable de maximiser la longueur des révolutions à au moins 5 ans, voire plus.

De plus, le recépage initial ne semble pas profiter au bilan énergétique des CCR et les résultats de cette étude ne confirment pas l'utilité d'une telle pratique pour augmenter les rendements, en particulier sur les sols sableux.

Enfin, particulièrement sur les sols argileux, le labour semble à proscrire. Toutefois, pour ameublir le sol en surface et mieux contrôler la compétition, effectuer un travail réduit à l'aide d'une charrue rotative à 5 cm de profondeur pourrait s'avérer être un bon compromis (Fontana *et al.* 2015).

ANNEXES

Table 5.1 Average CFN values of N, P, K, Ca, Mg and Mn, leaf mass (LM) and mean annual aboveground biomass yield for the 2011, 2012 and 2013 growing seasons combined. The coefficients of variation across growing seasons are reported in parentheses.

Sites	Concentrations of foliar nutrients									LM	Yield
	C	N	P	K	Ca	Mg	Mn	Fe	Zn		
	mg g-1						µg g^{-1}			mg leaf^{-1}	t ha^{-1} year^{-1}
ABI	45.8 (4%)	17.9 (8%)	1.6 (20%)	12.0 (25%)	17.0 (25%)	4.0 (19%)	1.77 (16%)	1.08 (109%)	1.30 (24%)	68 (2%)	10.1 (7%)
BEL	46.4	25.1	1.8	10.4	24.2	4.6	0.28	0.67	0.72	244	28.0
BOI	45.9 (4%)	19.2 (28%)	1.4 (30%)	12.0 (28%)	19.0 (23%)	4.2 (17%)	0.46 (12%)	0.48 (22%)	0.78 (14%)	129 (3%)	14.5 (16%)
HTG	44.8 (6%)	20.5 (17%)	1.6 (5%)	8.1 (11%)	20.4 (26%)	4.5 (6%)	0.29 (4%)	0.33 (22%)	0.86 (3%)	163 (6%)	21.9 (34%)
LAV	45.3 (4%)	17.0 (24%)	2.3 (20%)	22.6 (5%)	17.1 (37%)	1.2 (12%)	2.12 (18%)	0.65 (40%)	0.80 (14%)	91 (3%)	13.6 (12%)
MTL	45.6 (3%)	16.7 (5%)	1.6 (45%)	9.3 (10%)	16.6 (7%)	1.9 (15%)	2.28 (7%)	0.44 (19%)	2.73 (72%)	65 (1%)	7.7 (26%)
RXP	43.8 (2%)	24.5 (12%)	1.6 (8%)	5.5 (64%)	36.9 (1%)	3.7 (27%)	2.32 (23%)	0.45 (14%)	3.59 (114%)	122 (3%)	20.6 (12%)
SJPJ	45.8 (2%)	28.5 (25%)	2.2 (65%)	13.6 (15%)	24.1 (45%)	1.9 (18%)	1.52 (14%)	0.50 (46%)	1.23 (7%)	139 (11%)	23.8 (9%)
STR	44.7	17.8	1.4	19.6	24.4	1.9	1.56	0.33	1.02	72	9.9

No coefficient of variation is available at the BEL and STR sites because only one measurement was performed at each SRC.
SRCs belonging to the reduced database (n = 15) are indicated in bold.
ǂ2011 and 2013 only were used for calculation at SJPJ as stems were one year old in 2012.
‡2012 and 2013 only were available for calculation at HTG.

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